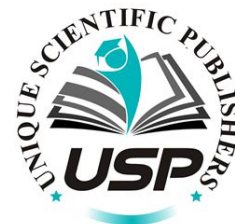




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## Contents

Assessing the Prospects and Challenges of Organic Agriculture in the Pothwar Region of Punjab, Pakistan .....	1
Muhammad Zeeshan Saeed, Hamza Hayat, Faraz Shafiq and Waqar-ul-Hassan Tareen	
Farmers' Awareness of Improved Maize Cropping Practices in Punjab, Pakistan .....	8
Muhammad Muddassir and Bader Alhafi Alotaibi	
Nutritional and Anti-nutritional Characteristics of Okra ( <i>Abelmoschus esculents</i> (L.) Moench) Accessions Grown in Pawe District, Northwestern Ethiopia .....	18
Misganew Andualem	
Assessment of Agronomic Traits of Plantain Cultivars and Hybrids Cultivated at High-Density Planting .....	22
N'guetta Adélaïde, Atsin Guy Joël Olivier, Aby N'goran, Traoré Siaka, Kouadio Demby Laetitia Muriel and Koffi Kouamé Germain Cyrille	
Potential of Biogas and Organic Fertilizers Production through Anaerobic Digestion of Slaughterhouse Waste in Ouagadougou, Burkina Faso .....	27
Soma Dohan Mariam, Pouya Bouinzemwendé Mathias, Zongo Nongma Armel, Kiba Delwendé Innocent, Gnankamary Zacharia and Sedogo Papoba Michel	
Cloning and Characterization of Trichoderma Glucanase Gene for Plant Transformation .....	31
Mawra Nadeem, Amar Ahmed Khan, Jawad Nadeem, Awais Ahmed Khan and Urooj Fatima	
Metabolomics, a Potential Way to Improve Abiotic Stresses Tolerance in Cereal Crops	47
Muhammad Usama Noman and Salman Azhar	
Utilisation of Herbal Bullets against Newcastle Disease in Poultry Sector of Asia and Africa (2012-2022) .....	56
Tayyaba Akhtar, Sana Shahid, Abeeha Asghar, Ifham Naeem, Shamreza Aziz and Tayyaba Ameer	
Assessment of Cassava Peel/Palm Kernel Cake Meal (PKM) on Growth Performance and Blood Parameters of Lactating Sows (Agricultural Extension Implication) .....	66
Moseri H, Umeri C, Onyemekonwu RC and Belonwu EN	



## Assessing the Prospects and Challenges of Organic Agriculture in the Pothwar Region of Punjab, Pakistan

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### ABSTRACT

Organic farming is vital in rural development and food production by promoting sustainable agriculture. The research study aimed to investigate the prospects and challenges of organic agriculture in Pothwar. For the research study, districts Chakwal, Rawalpindi and Islamabad were selected. 60 farmers from Chakwal, 60 from Rawalpindi, and 30 from Islamabad were chosen randomly for research for a sample size of 150 respondents. The interview schedule was prepared based on the literature review, and study objectives and interviews with the respondents were conducted in the native language. The majority, 64% of respondents, were owners of the land. The majority, 73.3% of respondents, use animals for land reclamation. 62% of respondents did not adopt a conservation tillage method. 74% of respondents implement crop diversification. 79% of respondents use the reforestation method. 81% of respondents have no knowledge or adoption of organic food safety. 94% respondents know cover crop manuring. The majority, 83% of respondents, know about organic farming, which is chemical and pathogen free. 82% of respondents know organic products with a high level of nutrients. The majority, 90% of respondents, know about organic farming increasing soil health. 58% of respondents have not adopted the organic certification guidelines. 51% of respondents have not adopted organic farming protection of soil. The majority 78% of respondents have not use bio fertilizer, a major component of organic farming. The majority, 77% of respondents, have adopted organic agriculture to increase soil health. Organic farming is not a better option for the Pothwar region of Punjab, according to 44.7% of respondents. 47% respondent agree that organic agriculture promotes biodiversity. 54% of respondents claimed that organic farming requires low input. 38% of respondents said that organic farming does not use machines. 34% of respondents strongly agree with a lack of input in organic farming. 41% of respondents agree less information about organic farming. 37% of respondents disagree that the production of organic farming is costly. Most organic farmers are aware of the requirements for organic certification, organic markets, the use of manure as fertilizer, the safety and quality of organic food, and the restricted variety of organic farming, but the admission rate of responders is meager. The benefits of organic agriculture include promoting biodiversity, increasing export possibilities, and lowering human health risks. High consumer demand for organic products, the need for few inputs in organic farming, and the aim of chemical-free agriculture are some supporting factors.

**Key words:** Prospect, Challenges, Organic Agriculture, Pothware Punjab Pakistan.

### INTRODUCTION

Organic farming promotes soil fertility, increases moisture retention, and protects the land from erosion and soil degradation losses. Under challenging circumstances, only diverse variants are utilized. Organic farming is a production method that prioritizes soil and ecosystem health more than harmful inputs and is suited to local needs. A complete production management method, organic agriculture encourages biodiversity, minimizes the use of pesticides, works in harmony with nature, and

integrates tradition, science, and innovation. It also improves biological cycles, soil biological activity, and agroecosystem health (Doris, Pierre, & Street, 2018)

In Pakistan, most large farms are operated by absentee property owners; in this case, the land is looked after by workers who have no sense of ownership over the production volume or profit margin. However, small landowners who work on farms and are constrained from purchasing substantial quantities of risky inputs must diversify their investments. To account for risks, they typically lower agricultural inputs. Removing inorganic

fertilizers, insecticides, and herbicides, as well as minimizing soil erosion, decreased production costs. They reduced production expenses by eliminating inorganic fertilizers, pesticides, and herbicides and reducing soil erosion (Friedmann, 2005).

Previous reports showed weed management in different crops through allopathy, such as canola (Diepenbrock, 2000) cotton (Finer & McMullen, 1990). Rice (Garris, Tai, Coburn, Kresovich, & McCouch, 2005) wheat and maize. Several potential allelopathic crops such as sunflower rice (Yu et al., 2002), sorghum brassica (Takasugi, Monde, Katsui, & Shirata, 1988), tobacco (*Nicotiana tabacum* L.), sesame (Anilakumar, Pal, Khanum, & Bawa, 2010) and trees such as eucalyptus (Rabelo Fernandes, Sampaio, Bilal, Lazo, & Fernandes, 2014) are known for having allelopathic effects. These plants contain allelochemicals such as benzoic acid, syringic acid, ferulic acid, gallic acid, protocatechuic acid, m-coumaric acid, caffeic acid, vanillic acid, hydroxy benzoic acid, p-coumaric acid, dhurrin, sorgoleone and hydroxybenzaldehyde whose extracts have been applied to suppress target weeds.

Sustainability is considered at the heart of organic agriculture (Smith et al., 2019) particularly from an economic perspective (Mughal & Faisal, 2021). Organic food is emerging as a combination of traditional and innovative food production, processing, and preservation methods with contemporary marketing practices (Tundys & Wiśniewski). The organic movement should be considered a valuable driving force toward sustainable food practices by transforming consumer values toward food quality through sustainable production methods (De Boer, Schösler, & Boersema, 2013). Therefore, it is crucial to provide the latest technologies, training, and awareness about organic agriculture to the farming communities (Anjum, Zada, & Tareen, 2016), especially for the survival of small farmers and to support their engagement with agriculture, to meet food security and livelihood issues (Mughal & Faisal, 2021).

The general problem of the study is to identify prospects and challenges of organic agriculture in Pothwar region of Punjab, Pakistan. This research has the following specific objectives. The study was limited to the responses of the organic agriculture farmer of the district Rawalpindi Chakwal and Islamabad. It was further limited to 150 respondents in the study area.

This research has the following specific objectives.

1. To assess the knowledge and adoption level of organic agriculture practices.
2. To identify the constraining and supporting factors of organic agriculture.

## MATERIALS AND METHODS

### Description of the Study Area

Rawalpindi is a district of Punjab, Pakistan. Rawalpindi. The community is located in the northernmost part of the Punjab province of Pakistan. Rawalpindi is the fourth large city in Pakistan. The Indus and Jhelum rivers are the main waterways, and the climate is renowned for its favorable health effects. Seven tehsils make up Rawalpindi's City District (Table 1).

The district has an area of 5,286 sq. km (2,041 sq. mi). 90 % of people in the room spoke Punjabi, talked by 4.3 % in Pashto, 3.5 % in Urdu, and 2.2 % in other languages.

**Table 1:** Rawalpindi Tehsils

No.	Tehsils of Rawalpindi
1	Gujar Khan.
2	Kahuta.
3	Kallar Syedan.
4	Kotli Sattian.
5	Murree.
6	Rawalpindi Tehsil.
7	Taxila

The district of Chakwal is in Punjab, Pakistan. Chakwal is primarily a rain-fed region. Chakwal is located in a semiarid, subtropical area. The district of Chakwal, in the northern part of Punjab, is surrounded by Khushab to the south, Rawalpindi to the north-east, Jhelum to the east, Mianwali to the west, and Attock to the northwest (table 2).

Pakistan's capital city, Islamabad, is situated in the Islamabad Capital Territory on the Pothohar Plateau, established between 1960 and 1970 to take Karachi's role as the nation's capital. President Ayyub Khan (1907–1974) was the one who created the idea of Islamabad as the nation's capital. Islamabad, Pakistan's capital, is situated on the Potohar Plateau northwest of the country. Being at the intersection between Rawalpindi and the North West Frontier Province has given this region historical significance.

**Table 2:** List of Chakwal Tehsils

No.	Tehsils of Chakwal
1	Chakwal.
2	Talagang.
3	Kallar Kahar.
4	Choa Saidan Shah.
5	Lawa.

### The Sampling of the Study

The task of collecting data relevant to the objective of the studies was challenging. Therefore, sampling was the only solution to solve the problem of getting information from a large area of the population. Consequently, it was decided to select a random sample for the study. Tehsil Rawalpindi Chakwal and Islamabad randomly selected organic agriculture growers for a sample size of 150 farmers.

### Instrument for Data Collection

Therefore, it was agreed to choose a random sample for the study. Rawalpindi, Islamabad, and Chakwal randomly selected farmers. Thirty farmers picked from Islamabad city. Sixty farmers were selected from Rawalpindi city and 60 from Chakwal. Purposefully selected organic farmer growers were chosen from each selected area to make a size of 150 respondents for the study. The interview schedule was built around open-ended and closed-ended questions to obtain accurate findings. The interview schedule was created in English and translated into Urdu for easy understanding of the respondents.

### Pre-Testing

Pre-testing is used to check the validity and authenticity of the interview schedule before the data collection. It is helpful in a way in which questions are being asked to get complete knowledge of the universe. It also enables the researcher to explore and identify the problem in the studied area. In addition, it also helps identify and discover the shortcoming in the scheduled questionnaire, and they may reschedule the questionnaire before collecting actual data.

To ensure data validity, the interview schedule was pre-tested on 30 randomly selected farmers with approximately the same characteristics as the actual farmer.

### Interviewing the Respondents

Researchers at their homes or farms personally interviewed the respondents. However, the questionnaire was prescribed in English, but the question was asked in Urdu. An extended time was spent with the respondent explaining the interview's purpose, clearing their doubts, and ensuring that the collected data would be used for education.

## RESULTS

The study's objective was to identify organ farming/agriculture growers' prospects and challenges in districts Rawalpindi, Chakwal and Islamabad.

### Knowledge of Organic Farming

86 % of farmers reduced chemical pesticides. 92 % of farmers use a grazing management method. The majority,

70.7% of respondents, did not use animals for land reclamation. 56.7 % of respondents use compost. 92% of respondents used conservation tillage. 89% of respondents implement crop diversification. 91% of respondents use the reforestation method. 93% of respondents know about organic food safety. It was discovered some practices of organic farming inquired and data complied as shown in Table 3.

### Adoption and Adoption Level

Food affordability, possession, allocation, and preferences all pertain to food availability (Muddassir & Al-Zahrani et al., 2022). Only a small portion of all studies on organic farming in developing countries is available in the scientific literature. Meanwhile, Bhattarai et al. (2013) also stated that government organizations can play important role in building a reliable supply chain in the context of developing countries. In developing nations like Pakistan, where the government promotes the procurement of organic goods for public sector organizations and nutrition programs through policy frameworks, government support is also notable in the development of organic farming and marketing (Edwardson & Santacoloma, 2013). 57% of respondents have not adopted crop rotation. 58% of respondents have not adopted the organic certification guidelines. 51% of respondents have not adopted organic farming protection of soil. 52% of farmers have adopted integrated pest management. 54% of respondents have no polyculture farming. The majority, 64% of respondents, have adopted windbreaks and shelterbelts, and 29% of respondents have medium-level use. Some statements of adoption and his level of organic farming practices are given in Table 4.

**Table 3:** Knowledge Abbot Organic Farming

Statement	Frequency	Yes %	Frequency	No%
cover cropping	141	94	9	6
Crop rotation	103	68.7	47	31.3
Organic certification guidelines	94	62.7	56	37.3
Organic farming soil protection	96	64	54	36
Integrated pest management	101	67.3	49	32.7
Polyculture farming	118	78.7	32	21.3
Biological control is better than pesticide	118	78.7	32	21.3
Windbreaks and shelterbelts	110	73.3	40	26.7
Organic farming regains soil nurturance	102	68	48	32
Precision agriculture	105	70	45	30
Organic marketing	97	64.7	53	35.3
Use bio-fertilizer	112	74.7	38	25.3
Usage of manure as fertilizer	136	90.7	14	9.3
Reduced chemical pesticide use	129	86	21	14
Grazing management	139	92.7	11	7.3
Use animals for land reclamation	38	25.3	112	74.7
Compost usage	85	56.7	65	43.3
Adopt the conservation tillage method	138	92	12	8
Implement the crop diversification	134	89.3	16	10.7
Reforestation method	137	91.3	13	8.7
Organic food safety	140	93.3	10	6.7
Quality of organically grown crops	104	69.3	40	30.7
Chemicals and Pathogens free	125	83.3	25	16.7
Bio-fertilizer is a significant component of organic farming	121	81	29	19
Organic products have high levels of nutrients	124	82.7	20	17.3
Organic farming has a limited variety of crop	134	89.3	16	10.7
Organic farming support biodiversity	126	84	24	16
Organic farming increase soil health	135	90	15	10

### Prospects of Organic Farming

This question asked respondents about the prospects of organic farming. Five grading seals were produced when respondents were questioned about the organic agricultural opportunities. The information obtained from the responders is shown in Table 5. An important part of Pakistan's national economy is agriculture. Its geographic and ecological heterogeneity provides competitive advantage for growth of important crops, fruits, and vegetables throughout the year (Musa, Program, & Gurung, 2015) notably naturally or by default organic across the various locations (Anjum et al., 2016). In Pakistan, there are approximately 1.51 million hectares of organic land as opposed to 22.6 million hectares of inorganic land (Musa et al., 2015). However, Pakistan has only 45,299 ha of globally certified organic agriculture land, which is significantly less than other Asian nations and only 0.1 percent of the total land area, despite having vast naturally organic regions (Willer, 2018). While a significant amount of food that has been certified as organic is sold on international markets.

### Supporting Factors

In organic farming, 34% agreed that there is a high demand for organic agriculture. 54% of respondents agree that organic farming requires low input. 43% of respondents agree organic agriculture aims to stop the use of chemicals. 36% of respondents agree, and 29% moderately recycle animal waste. 38% of respondents agree that organic farming does not use machines. 33% of respondents disagree that genetically modified organisms are not grown. 44% of respondents moderated that the government should provide subsidies for organic farming. Respondents were asked about the supporting factors for organic agriculture and given five rating scales. The data gathered from the respondents was compiled and given in Table 6. One of the main causes of Pakistan's unorganized organic food business is the lack of organic food rules, which are currently being developed (Lernoud & Willer, 2017). Therefore, Pakistan's organic food industry and legislation are challenging, much like in many other countries. Certain regional nations, like Bangladesh, Sri Lanka, and even some African governments, including Kenya, Uganda, and Rwanda, have more recently enacted organic rules with appropriate certification and labelling processes (Willer, 2018). Organic farming has both private and public benefits, which indicate that it should be encouraged to support sustainable farming methods (Husnain, Khan, & Mahmood, 2017). The system of organic food production and agriculture is.

### Challenges and Problem

Respondents to this question indicate the challenges with organic farming. The respondents were inquired about challenges and problems they have faced while performing organic production on a given scale, and the results in Table 7.

### Knowledge of Organic Farming Practices

The majority, 94% of respondents, know cover crop/green manuring. 68.7% of respondents know crop rotation. 62.7% of respondents know organic certification guidelines. The majority, 64% of respondents, know

organic farming's soil protection. 67.3% of farmers' knowledge of integrated pest management. 78.7% of respondents know about polyculture farming. The majority, 73.3% of respondents, knew about windbreaks and shelterbelts. 68% of respondent's knowledge of organic farming regained the soil's nurturance. Biological control is better than pesticides. 78% of respondents know. The majority of respondents know precision agriculture. 64.7% of respondents know organic markets. 74% of respondents use bio-fertilizer. The majority of 90 % of respondents use manure as fertilizer. 86 % of farmers reduced chemical pesticides. 92 % of farmers use a grazing management method. The majority, 70.7% of respondents, did not use animals for land reclamation. 56.7% of respondents use compost. 92% of respondents used conservation tillage. 89% of respondents implement crop diversification. 91% of respondents use the reforestation method. 93% of respondents know about organic food safety. 69% of respondents know the quality of organically grown crops. The majority, 83% of respondents, know about organic farming, which is chemical and pathogen free. The majority, 81% of respondents, know bio-fertilizers as a significant component of organic farming. 82% of respondents know organic products with a high level of nutrients. 89.3% of respondents know about organic farming and have a limited variety of crops. 84% of respondents know organic farming's support for biodiversity. The majority, 90% of respondents, know about organic farming increasing soil health.

### Adoption and Adoption Level of Organic Farming Practices

The majority, 78% of respondents, have adopted cover crop/green manuring, and 41% of respondents have a medium level of adoption. 57% of respondents have not adopted crop rotation. 58% of respondents have not adopted the organic certification guidelines. 51% of respondents have not adopted organic farming protection of soil. 52% of farmers have adopted integrated pest management. 54% of respondents have no polyculture farming. The majority, 64% of respondents, have adopted windbreaks and shelterbelts, and 29% of respondents have medium-level use. 56% of respondents did not adopt organic farming to regain the soil's nurturance. Biological control is better than pesticides. 51.3% of respondents have not adopted. Precision agriculture is used by the majority of respondents (50.7%). 58% of respondents have not adopted the organic market. 54% of respondents use bio-fertilizer. The majority of 90 % of respondents use manure as fertilizer. Eighty-five % of farmers reduced their use of chemical pesticides. Grazing management is used by 86% of farmers. The majority, 73.3% of respondents, Use animals for land reclamation. 69.3% of those polled do not use compost. 62% of respondents did not adopt a conservation tillage method. 74% of respondents implement crop diversification. 79% of respondents use the reforestation method. 81% of respondents have no knowledge or adoption of organic food safety. 72.3% of respondents have adopted the quality of organically grown crops. The majority of respondents (80%) believe that organic farming is free of chemicals and pathogens. Majority 78% of respondents have no

adoption of bio fertilizer, a major component of organic farming.80% of respondents have adopted organic products with a high level of nutrients.79% of respondents who have adopted organic farming have a limited variety of crops.86% of respondents have adopted organic farming to support biodiversity. The majority, 77% of respondents, have adopted organic agriculture to increase soil health.

#### Prospect and Prospect Level of Organic Farming

38% of respondents said that healthy nutrients. 39.3% of respondents agree that being environmentally friendly is essential. Bather for soil health received 35% moderate responses.

38% of respondents had no answer about marketing demand. Thirty respondents had no answer about export potential.33.3% of respondents strongly agree that conventional methods are more profitable.36% agree with

reducing human health risks.40% of farmers agree that marketing. 40% of moderate farmers are insect pest resistant.43% agree that low technology level is a problem. After the Lon version, there was a 26% no-answer high yield.30% agree that organic is safe and pure. Organic farming is not a better option for the Pothwar region of Punjab, according to 44.7% of respondents.47% agree that organic agriculture promotes biodiversity.

#### Support factors of organic farming

34% agreed that there is a high demand for organic farming.54% of respondents agree that organic farming requires low input.43% of respondents agree organic farming aims to stop the use of chemicals.36% of respondents agree, and 29% moderately recycle animal waste.38% of respondents agree that organic farming does not use machines.33% of respondents disagree that genetically modified organisms are not grown.44% of

**Table 4:** Adoptions and Adoption Level

Statement	F	Y %	F	N %	F	N %	F	L %	F	M%	F	H%
cover cropping	117	78	33	22	31	20.7	33	22	62	41.3	24	16
Crop rotation	64	42.7	86	57.3	86	57.3	23	15.3	33	22	8	5.3
Organic certification guidelines	62	41.3	88	58.7	88	58.7	11	7.3	12	8	39	26
Organic farming soil protection	73	48.7	77	51.3	80	51.3	26	18.3	9	7	35	23.4
Integrated pest management	79	52.7	71	47.3	71	47.3	27	18	38	25.3	14	9.3
Polyculture farming	69	46	81	54	81	54	20	13.3	20	13.3	29	19.3
Biological control is better than pesticide	96	64	54	36	54	36	21	14	44	29.3	31	20.7
Windbreaks and shelterbelts	66	44	84	56	84	56	16	10.7	29	19.3	21	14
Organic farming regains soil nurturance	73	48.7	77	51.3	77	51.3	31	20.7	21	14	21	14
Precision agriculture	76	50.7	74	49.3	74	49.3	17	11.3	3	2	29	19.3
Organic marketing	87	58	63	42	63	42	30	20	15	10	40	26.7
Use bio-fertilizer	82	54.7	68	45.3	68	45.3	7	4.7	38	25.3	37	24.6
Manure fertilizer	128	85.3	22	14.7	22	14.7	58	38.3	34	22.7	36	24
Reduced chemical pesticide use	130	86.7	20	13.3	20	13.7	79	52.7	37	24.7	13	8.7
Grazing management	110	73.3	40	26.7	40	26.7	68	45.3	29	19.3	13	8.7
Use animals for land reclamation	46	30.7	104	69.3	104	69.3	25	16.6	13	8.3	8	5.3
Compost usage	57	38	93	62	93	62	19	12.6	23	15.3	15	10
Adopt the conservation tillage method	112	74.7	38	25.3	44	29.3	29	19.3	32	21.3	45	30
Implement the crop diversification	119	79.3	31	20.7	31	20.3	70	46.3	35	23.3	14	9.3
Reforestation method	28	18.7	122	81.3	22	81.3	11	7.3	11	7.3	6	4
Organic food safety	109	72.7	41	27.3	34	22.7	68	45.3	29	19.3	19	12.7
Quality of organically grown crops	120	80	30	20	29	19.3	70	46.7	27	18	24	16
Chemicals and Pathogens free	117	78	33	28	30	20	65	43.3	36	24	19	12.7
Bio-fertilizer is a significant component OF	121	80.7	29	19.3	32	21.3	49	32.7	4	26.7	29	19.3
Organic products have a high level of nutrients	119	79.3	31	20.7	31	20.7	49	32.7	47	31.7	23	15.3
OF have a limited variety of crop	130	86.7	20	13.3	20	13.3	40	26.7	46	30.7	44	29.3
OF support biodiversity	126	84	24	16	27	18	59	39.3	27	18	37	24.7
OF increases soil health	116	77.3	34	22.7	34	22.7	32	21.3	62	41.3	22	14.7

F = Frequency, Y = Yes, N = No, L = Low, M = medium, H = High OF = organic farming.

**Table 5:** Prospects of Organic Farming

Prospects	F	SD%	F	D%	F	M%	F	A%	F	SA%
Healthy nutrients	6	4	15	10	36	24	57	38	36	24
Environmentally friendly	2	1.3	13	8.7	42	28	59	39.3	34	22.7
Bather for soil health	3	2	8	5.3	53	35	44	29.3	42	28
Marketing demand	2	1.3	40	26.7	58	38.7	37	24.7	13	8.7
Export potential	19	12.7	19	12.7	46	30.7	39	26	27	18
More profitable than conventional	23	15.3	32	21.3	50	33.3	43	28.7	2	1.3
Reduce human health risks	2	1.3	3	2	56	37.3	55	36.7	34	22.7
Require Marketing	2	1.3	4	2.7	41	27.3	61	40.7	42	28
Resistant to insect pest	5	3.3	39	26	60	40	45	30	1	7
Low technology level	1	7	2	1.3	53	35.3	65	43.3	29	19.3
High yield after Lon version period	5	3.3	20	13	40	26.7	34	22.7	51	34
Safe and pure	21	14	20	13.3	45	30	37	24.7	27	18
Organic farming is a better option for the Pothwar region of Punjab	2	1.3	41	27.3	67	44.7	38	25.3	2	1.3
Organic agriculture promotes biodiversity	1	7	24	16	26	17.3	61	40.7	38	25.3

F = Frequency, SD = Strongly Disagree, D = Disagree, M = Moderate, A = Agree, SA = Strong Agree.

**Table 6: Supporting Factors of Organic Farming**

S.F	F	SD%	F	D%	F	M%	F	A%	F	SA%
High demand for OF	4	2.7	6	4	44	29.3	51	34	45	30
OF require low input	2	1.3	15	10	54	36	52	34.7	27	18
OF aims to stop the use of chemicals	1	7	24	16	44	29.3	62	43	19	12.7
Recycle the waste of animal	1	7	3	2	54	36	44	29.3	48	32
OF not use machines	8	5.3	53	21.3	47	31.3	58	38.7	5	3.3
GMO	9	6	50	33.3	52	34.7	30	20	9	6
Govt. give the sport	12	8	36	24	66	44	35	23.3	1	7
Increase the resilience of climate	3	2	17	11.3	48	32	60	40	22	14.7
Higher profit in OF	61	40.7	49	32.7	1	3	1	3	1	7
Stop health problems	11	7.3	16	10.7	40	26.7	55	36.7	28	18.7
NGOs support	68	45.3	47	31.3	20	13.3	6	4	9	6

S.F = Supporting Factors, F = frequency, SD = Strongly Disagree, D = Disagree M = Moderate, A = Agree, SA = Strong Agree, OF = Organic Farming, GMO = Genetically Modified Organism, Govt. = Government, NGOs = Non-Governmental Organization.

**Table 7: Challenges and Problems**

Challenges in OF	F	SD%	F	D%	F	M%	F	A%	F	SA%
Low production	3	2.0	18	12.0	23	15.3	55	36.7	51	34
Lack of input	3	2	4	2.7	37	24.7	54	36	52	34.7
Less information OF	3	2	4	2.7	24	16	62	41.3	57	38
Soil fertility	8	5.3	53	35.3	43	28.3	37	24.7	9	16
Costly production	52	37.7	56	37.3	37	24.7	3	2	2	1.3
Small land holding	9	6	17	11.3	39	26	43	28.7	42	28
Poor infrastructure	1	7	13	8.7	31	20.7	58	38.7	47	31.3
Govt. support	2	3	1	7	36	24	71	47.3	36	24
Lack training program	4	2.7	7	4.7	25	16.7	77	51.3	37	24.7
More disease attack	4	2.7	31	20.7	47	31.3	63	42	5	3.3
Less disease-resistant varieties	6	4	7	4.7	28	18.7	57	38	52	34.7
High-production seed short	2	1.3	21	14	32	21.3	60	40	35	23.3
Less demand in the domestic market	45	30	56	37.3	31	20.7	17	11.3	1	7
High organic product price	4	2.7	24	16	35	23.3	66	43.3	21	14
Labor intensive	17	11.3	28	18.7	29	19.3	46	30.7	30	20
Pest attack	11	7.3	12	8	32	21.3	57	38	38	25.3
Less availability of organic fertilizer	5	3.3	24	16	42	28	49	32.7	30	20

OF = Organic Farming, F = Frequency, SD = Strongly Disagree, D = Disagree, M = Moderate, A = Agree, SA = Strong Agree, Govt. = Government.

respondents moderated that the government should provide subsidies for organic farming. 40% of respondents agree that increasing the resilience of the organic farming is not more profitable, according to 60% of respondents. 37% of respondents did not agree that organic farming stops health problems. 45% do not agree, and 31% moderate about NGOs' support for organic farming.

### Concluding Remarks

Various demographic and socio-economic variables of the respondents, like education, age, landholding, ownership of the land, and marital status, seem to play a role in organic farming, as evident from the study that more than half of the respondents were, educated, young, and married, Owner-cum-tenant, experienced and smallholder were actively engaged in organic farming. They were full-time farmers and had access to the local market. Livestock rearing and raising crops were their primary source of income for them.

Organic farmers mainly utilize confidential sources of information and fellow farmers to fulfilling their knowledge requirements regarding organic farming and possess little knowledge about organic farming.

Most organic farmers are aware of the requirements for organic certification, organic markets, the use of manure as fertilizer, the safety and quality of organic food, and the restricted variety of organic farming, but the admission rate of responders is meager. The benefits of

organic agriculture include promoting biodiversity, increasing export possibilities, and lowering human health risks. High consumer demand for organic products, the need for few inputs in organic farming, and the aim of chemical-free agriculture are some supporting factors. Low production, a lack of information, a lack of organic markets, a lack of government backing, and a limited supply of organic fertilizer are the obstacles organic farming faces. The implementation of organic farming is hampered by a lack of knowledge, training, better varieties, extension services, decision-making, and access.

The agriculture extension department played very little involvement in organic farming. Cultural barriers make it challenging to discuss organic farming. There was no training or counseling on how to produce organic food.

The extension department should bind extension workers to perform their duties by visiting farming communities regularly, and their monitoring should be done. It improved training programmers on organic farming. Give an account of organic farming. The government gives support for organic farming and subsidy. Farmers should use biofertilizers.

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## Farmers' Awareness of Improved Maize Cropping Practices in Punjab, Pakistan

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### ABSTRACT

Study was aimed to assess awareness of improved maize cropping practices among maize farmers. The data was collected by using simple random sampling and 120 maize farmers were involved in the study. A structured interview was used for Personal interview. The study revealed that 84.2% of the farmers were aware of improved maize cropping practices. The farmers who had been to college and higher education, large scale farmers and farmers who were visiting extension department were aware of improved maize practices. The significant difference was found regarding overall awareness among farmer's groups including farmers who had been to college and higher education and who had been to high school or less than high school, farmers who were visiting extension department and who were not visiting extension department, small- and large-scale farmers (0.00,  $P > .001$ ). Practice-wise only seed treatment, weedicide and harvesting methods did not show significant differences. The college and higher education, large scale farmers and visit to extension department were the determinants of awareness among farmers. The government should implement extension trainings and awareness programs to create awareness among small scale farmers and farmers and who had high school education or less than high school and farmers who were not visiting extension department.

**Key words:** Awareness 2, Agricultural extension 3, Cropping practices maize 4, Pakistan

### INTRODUCTION

Pakistan is essentially an agricultural country and its economy largely depends on the bumper harvest. Agriculture is the main source of foreign exchange earnings for the country. It's a main sector of the economy as it also provides raw materials to main industrial units of the country and a major share of exports also come from agriculture. It accounts for 19.8% of the Gross Domestic Product (GDP) and it remains by far the largest employer absorbing 42.3% of the country's total labour force (Go, 2016). Maize (*Zea Mays L*) is one of the main crops after wheat and rice which has important place in the economic development of Pakistan. It contributed about 0.4 % to GDP and 2.1 % to the value added in agriculture (Go, 2016). Agricultural development could be achieved by diffusion of information about improved practices through effective communication. Application of improved agricultural technologies increase farm production and subsequently enhancing socioeconomic status of the farmers (Ani *et al.*, 2004). Awareness of improved cropping practices among farmers stimulate their interest towards adoption of the latest agricultural technologies. In developing countries, governments are conducting

agricultural programs by agricultural extension agencies for increasing farmers' awareness (Swanson *et al.*, 2008). In Pakistan, agricultural extension services play a vital role in increasing crop production by educating farmers about improved cultural practices; proper seed requirement, timely sowing, balanced and efficient use of fertilizer, efficient use of irrigation methods, pesticide use, effective crop management, harvesting, threshing and storage and marketing. Various extension teaching methods are being used to educate farmers for example, training sessions and different individual, group, and mass extension methods (Shahbaz & Ata, 2014). The agricultural production in Pakistan ranges between 29% and 52%, far lower than the world's best averages for maize, Cotton, Wheat, Sugar cane crops (Muddassir *et al.*, 2020). The reason behind the low production is the lack of adoption of improved and innovative practices. The low yield reflects that some technological gap exists in the awareness of improved maize cropping practices among the farmers. The limited systematic effort has been made to assess the causes of the low production of the maize crop. Keeping the above points in mind, the specific objective of the study was to analyse the farmers' awareness of improved maize cropping practices in the study area.

## MATERIALS AND METHODS

### Location of Study

The quantitative research was conducted in the District Jhang, Punjab as shown in Fig. 1. According to the census 2017, the district population was 2.743 million, of which 2.145 million lived in rural areas. Almost all the area except some area turn rocky. Tehsil Jhang is a subdivision (Tehsil) of District Jhang in the Punjab province of Pakistan. The neighbor districts are Chiniot, Faisalabad, Multan, Sargodha, Layyah, and Sahiwal.

### Selection of Unions

The Tehsil Jhang consists of 55 Union Councils (UC). Fifteen unions are urban and 40 are rural. Six rural union councils were randomly selected to represent the maize farmers' population in the study area.

### Selection of Villages

Each union council contains approximately 6 villages; one village was selected from each UC through random number technique.

### Selection of Respondents

The population in the six villages was reached about 1200 farmers (Agricultural Directorate of Punjab, 2017). To capture the information from farmers, a sample size of 120 was selected to represent 10% of the universe using a simple random sampling method.

### Data Collection

A questionnaire was developed, pretested and validated. Questions were asked in local language for the convenience of interviewees to get the required information with maximum accuracy. The validity and reliability of the interview schedule were pre-tested on 20 respondents. As a result of pre-testing, necessary amendments were incorporated to give it a final shape. Reliability of attitudes scale was tested by calculating the Cronbach's Alpha test ( $\alpha=0.81$ ). The index of improving maize practices was obtained from some latest extension pamphlets on maize in Pakistan. This index was reviewed from five extension workers in the study area. The extension workers have expressed the degree of approval of each practice (perfectly OK, OK, to some extent, not OK), with the ability to add amendments that it was deemed appropriate for each practice, as well as delete practices that it deemed inappropriate, and on the other hand ability to add new practices recommended in the study area. This resulted in 30 practices representing the index of improving maize practices.

### Independent Variables

Variables of education, Land area and farmers visits to extension department of the respondents were used as independent variables.

### Dependent Variables

The awareness of respondents was used as dependent variables. The measurement scale of awareness was (aware and unaware). The survey was used to standardize the awareness level. The respondents who respond according to the recommended improved maize cropping practices

were considered aware and who respond according to traditional practices were considered as unaware.

### Data Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) program. Descriptive statistics such as frequencies, percentages, mean and standard deviation was used address the research objective. Mann-Whitney U test was used appropriately to compare differences between two independent variables against ordinal variables.

## RESULTS

### Farmers' Profile

Summary of demographic characteristics of farmers is shown in Table 1. The findings show that 65% of the farmers were belonged to age category 30-40 years, 17.5% belonged to 25-30 years, 15.8% belonged to 40-50 years and 1.7% were belonged to 50-60 years' age category. Regarding farming experience 90.8% of the farmers were having Low farming experience and 9.2% of the farmers were having medium experience. Maximum number of farmers 63.3% were small scale famers and 36.7% of them were large scale farmers. and more than half of the farmers 59.2% had been to higher school and less than higher school and 40.8% of the farmers had been to college and higher education. In term of extension agent's visits to farmers, 93.3% extension agents visited to farmers at their farm fortnightly, 5.8% and 0.8% extension agent visited to farmers once in a six months and once in a more than six months respectively.

### Farmers Source of Information

Table 2 showed that 65% of the farmers visited extension department during crop season and 64.2% of the farmers followed their instructions regarding cropping technology. Other than extension department, 31.7% of the farmers were getting information from progressive farmers, 16.7%, 1.7% and 1.7% of the farmers were relied print media, electronic media and neighbor farmers respectively, but farmers were highly relying on extension agents 48.3% for information.

### Overall Mean and SD

Table 3 shows the awareness level of famers about improved maize cropping practices. The overall awareness among farmers reported high mean= 2.68 and SD= 0.73. The detail of each cropping practices is mentioned below.

The Fig. 2 shows the awareness of improved maize practices among farmers. The respondents of the study were aware of land preparation 46.7%, fertilization 43.3% irrigation 66.7%, sowing time 54.2%, sowing methods 54.2%, intercropping practices 55.8%, seed rate 92.5%, seed varieties 83.3%, seed treatment 98.3%, plant protection 93.3%, plant diseases 92.5%, weedicide 98.3% and harvesting 100%. However, in the Table 3 the respondents were unaware regarding land preparation by 3-4 times intercrossing harrowing by planking with each plough and mean was =1 with SD=0.00. Fertilization at the time of sowing DAP 2.5 bags/acre, SOP 1 bag/acre, Urea 2.5 bag/acre and mean was=1.86 with SD=0.99.



Fig. 1: Map of the geographical area

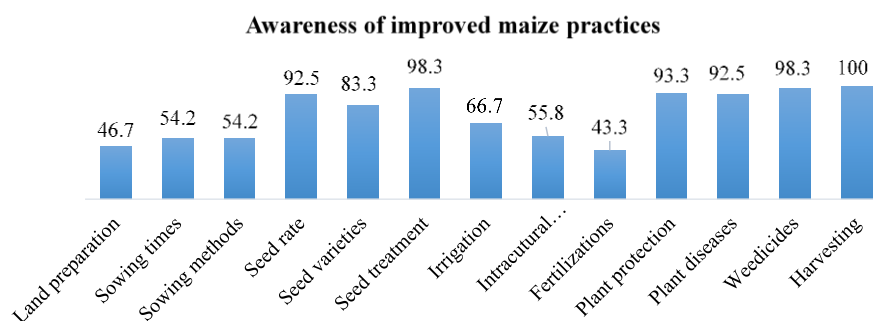


Fig. 2: Overall awareness of improved maize cropping practices.

Table 1: Demographic profile of farmers.

Variables	Frequency	Percent
Age (n=120)		
25-30 years	21	17.5
30-40 years	78	65.0
40-50 years	19	15.8
50-60 years	2	1.7
Farming experience (120)		
Low experience	109	90.8
Medium experience	11	9.2
Land area (n=120)		
Small scale farmer	76	63.3
Large scale farmer	44	36.7
Education (n=120)		
Higher school and less than higher school	71	59.2
College and higher education	49	40.8
Cultivated area (n=120)		
Less than 5 acres	12	10.0
5-7 acres	34	28.3
7-9 acres	38	31.7
9-11 acres	30	25.0
more than 11 acres	6	5.0
Extension agent visits to farmers (n=120)		
once in more than 6 months	1	0.8
once in 6 months	7	5.8
fortnightly	112	93.3

### Overall Awareness

Table 4 shows percentage of reported aware and unaware farmers about improved maize cropping practices. Results demonstrated that more than eighty percent farmers 84.2% were aware about maize cropping practices and only 19% of the farmers were unaware.

### Mean Rank of Awareness of Improved Maize Practices According to Education

Awareness scores of farmers who had been to high school and less than high school and who had been to college or higher education was compared and Mann-Whitney U test was used to test the significant difference

Table 2: Source of information and application of extension recommendations.

Variables	Frequency	Percent
Do you visit agricultural extension department during crop's problem (n=120)		
No	42	35.0
Yes	78	65.0
Do you follow extension recommendations (n=120)		
No	43	35.8
Yes	77	64.2
Source of information (n=120)		
Neighbor farmer	2	1.7
Progressive farmer	38	31.7
Print media	20	16.7
Electronic media	2	1.7
Extension agent	58	48.3

between two farmer's groups regarding their education. According to Table 5, the farmers who had been to college and higher education were aware about all improved maize cropping practice mentioned in the Table 5 and other farmers were not aware who had been to high school and less than high school.

### Mann-Whitney U Test Results of Awareness of Improved Maize Practices According to Education

The difference between two farmer's groups was statistically significant at  $p < 0.001$ . The awareness of improved maize practices among farmers who had been to college and higher education and who had been to high school or less than high school about each practice showed significant differences in Table 6 except seed rate, seed treatment, plant protection, plant disease, weedicide and harvesting methods.

### Overall Awareness Mann-Whitney U Test Result

Table 7 shows the comparison of overall awareness of famers according to their education; statistically significant difference was found with the P value=0.000.

**Table 3:** Farmer's awareness of recommended maize cropping practices

Variables	Mean	SD
Land preparations		
3-4 times intercrossing harrowing by planking with each plough	1.00	0.00
3-4 times ploughing followed by planking	1.93	1.00
Rotavator	1.93	1.00
Sowing times		
Mid-February to Mid-March and July and August for seed	2.08	1.00
Mid-February to Mid-March and July and August for fodder	2.08	1.00
Sowing methods		
Single row cotton drill	2.08	1.00
Maize planter (R-R= 75 and (P-P=25cm)	2.10	0.99
Seed varieties		
Sgd.2002	2.66	0.74
YH-1898	2.68	0.73
Super green maize	2.70	0.71
Seed rate		
Ridge sowing 8-10 Kg per acre	2.85	0.52
Seed drill (12 15kg/acre)	2.85	0.52
Broadcasting (40-50 kg per acre)	2.85	0.52
Seed treatment		
Imidacloprid WS 70 (5-10g per Kg)	2.96	0.25
Irrigation		
At sowing time	2.76	0.64
After germination of plant	2.73	0.68
Vegetative Phase (3 irrigations)	2.51	0.85
Flowering Phase (2 irrigations)	2.33	0.94
Maturity Phase (1 irrigation)	2.25	0.97
Intracultural practices		
2-3 Hoeing	2.11	0.99
Thinning when the crop is 20cm in height	2.16	0.99
Chemical fertilizers		
At the time of sowing DAP (2.5 bags/acre), SOP (1 bag/acre) Urea (2.5 bag/acre)	1.86	0.99
When plant height becomes 1.5 feet Urea (1 bags/acre)	2.70	0.71
When plant height becomes 2.5 to 3 feet Urea (1bag/acre)	2.78	0.62
At flowering stage Urea (1 bags/acre)	2.88	0.47
Plant protection		
Maize Stem Borer: Carbofuran 3G (6kg per acre)	2.86	0.50
Maize Stem Borer: Fipronil (8kg per acre)	2.88	0.47
Shoot Fly: Imidacloprid 200SL (200ml/acre)	2.95	0.31
Aphid & Jassid: Imidacloprid (200ml/acre)	2.93	0.36
Plant Diseases		
Seedling Rot: Thiophenate Methyl 3-4 gm/kg (Topsin) or Benlate (2g/kg)	2.88	0.47
Leaf Blight/stalk root disease: Dithene (1kg@400 L of water) or Azomide (azoxystrobin 250SC (25% w/v)	2.85	0.52
Weedicides		
Itset, Bhakra and Karand: Atrazine38SC (330- 400ml/acre or Pendimethaline (1litre/acre)	2.96	0.25
Jangli Hallow: Pendimethaline and Metolachlor (1litre/acre)	2.96	0.25
Dela, Madhana: Click 72.4SE (Atrazine + Acetochlor) 600ml/acre	3.00	0.00
Harvesting		
Manual harvesting: Spring crop (June); Autumn crop (Mid November)	3.00	0.00
Shelling: Spring crop (June) Autumn crop (Mid November)	3.00	0.00
Overall	2.68	0.73

**Table 4:** Classification of farmers according to their awareness about improved maize cropping practices.

Awareness categories	Frequency (n=120)	%
Aware	101	84.2
Unaware	19	15.8

The farmers who had been to college or higher education were aware of improved maize practices. Other farmers who had been to high school or less than high school were unaware.

#### Mean Rank of Awareness of Improved Maize Practices According to Land Area

Awareness scores of small-scale farmers and large-scale farmers was compared and Mann-Whitney U test was

used to test the significant difference between two farmer's groups regarding their land area. According to Table 8, large scale farmers were aware about all improved maize cropping practices mentioned in the Table 8 and other small farmers were not aware.

#### Mann-Whitney U Test Results of Awareness of Improved Maize Practices According Land Area

Table 9 demonstrated the difference between two farmer's groups was statistically significant at  $p < 0.001$ . Regarding the awareness of all improved maize practices among small and large farmers showed significant differences except seed rate, seed treatment, plant protection, plant disease, weedicide and harvesting methods.

**Table 5:** Mann-Whitney U test with mean rank

Awareness of improved maize practices According to education	Ranks		N	Mean Rank	Sum of Rank
	Groups				
Land preparation	Higher school and less than higher school	71	38.42	2727.50	
	College and higher education	49	92.50	4532.50	
	Total	120			
Sowing time	Higher school and less than higher school	71	41.52	2948.00	
	College and higher education	49	88.00	4312.00	
	Total	120			
Method of sowing	Higher school and less than higher school	71	41.52	2948.00	
	College and higher education	49	88.00	4312.00	
	Total	120			
Seed rate	Higher school and less than higher school	71	57.39	4075.00	
	College and higher education	49	65.00	3185.00	
	Total	120			
Seed treatment	Higher school and less than higher school	71	59.81	4246.50	
	College and higher education	49	61.50	3013.50	
	Total	120			
Seed varieties	Higher school and less than higher school	71	53.60	3805.50	
	College and higher education	49	70.50	3454.50	
	Total	120			
Irrigations	Higher school and less than higher school	71	46.70	3315.50	
	College and higher education	49	80.50	3944.50	
	Total	120			
Intracultural practices	Higher school and less than higher school	71	42.21	2997.00	
	College and higher education	49	87.00	4263.00	
	Total	120			
Fertilization	Higher school and less than higher school	71	37.04	2629.50	
	College and higher education	49	94.50	4630.50	
	Total	120			
Plant protection	Higher school and less than higher school	71	57.74	4099.50	
	College and higher education	49	64.50	3160.50	
	Total	120			
Plant diseases	Higher school and less than higher school	71	57.39	4075.00	
	College and higher education	49	65.00	3185.00	
	Total	120			
Weedicides	Higher school and less than higher school	71	59.81	4246.50	
	College and higher education	49	61.50	3013.50	
	Total	120			
Harvesting	Higher school and less than higher school	71	59.81	4246.50	
	College and higher education	49	61.50	3013.50	
	Total	120			

**Table 6:** Mann-Whitney U test results according to education

Awareness according to education	Mann-Whitney U	Wilcoxon W	z	P value
Land preparation	171.50	2727.50	-9.688	0.00
Sowing time	392.00	2948.00	-8.336	0.00
Sowing methods	392.00	2948.00	-8.336	0.00
Seed rate	1519.00	4075.00	-2.580	0.01
Seed treatment	1690.50	4246.50	-1.180	0.23
Seed varieties	1249.50	3805.50	-4.053	0.00
Irrigations	759.50	3315.50	-6.408	0.00
Intracultural practices	441.00	2997.00	-8.060	0.00
Fertilizations	73.50	2629.50	-10.363	0.00
Plant protection	1543.50	4099.50	-2.422	0.01
Plant diseases	1519.00	4075.00	-2.580	0.01
Weedicides	1690.50	4246.50	-1.180	0.23
Harvesting	1739.50	2964.50	.000	1.00

**Table 7:** Comparison of overall awareness of farmers according to their education

Groups	N	Mean Rank	Sum of Rank
Higher school and less than higher school	71	53.94	3830.00
College and higher education	49	70.00	3430.00
Total	120		
Categories			Awareness
Mann-Whitney U			1274.00
Wilcoxon W			3830.00
Z			-3.931
P value			0.00

**Table 8:** Mann-Whitney U test with mean rank

	Rank			
Awareness according to farmers scale	Farmers scales	N	Mean Rank	Sum of Ranks
Land preparation	Small scale farmer	76	41.97	3190.00
	Large scale farmer	44	92.50	4070.00
	Total	120		
Sowing time	Small scale farmer	76	44.58	3388.00
	Large scale farmer	44	88.00	3872.00
	Total	120		
Sowing methods	Small scale farmer	76	44.58	3388.00
	Large scale farmer	44	88.00	3872.00
	Total	120		
Seed rate	Small scale farmer	76	57.89	4400.00
	Large scale farmer	44	65.00	2860.00
	Total	120		
Seed treatment	Small scale farmer	76	59.92	4554.00
	Large scale farmer	44	61.50	2706.00
	Total	120		
Seed varieties	Small scale farmer	76	54.71	4158.00
	Large scale farmers	44	70.50	3102.00
	Total	120		
Irrigations	Small scale farmer	76	48.92	3718.00
	Large scale farmer	44	80.50	3542.00
	Total	120		
Intercultural practices	Small scale farmer	76	45.16	3432.00
	Large scale farmer	44	87.00	3828.00
	Total	120		
Fertilizations	Small scale farmer	76	40.82	3102.00
	Large scale farmer	44	94.50	4158.00
	Total	120		
Plant Protection	Small scale farmer	76	58.18	4422.00
	Large scale farmer	44	64.50	2838.00
	Total	120		
Plant diseases	Small scale farmer	76	57.89	4400.00
	Large scale farmer	44	65.00	2860.00
	Total	120		
Weedicide	Small scale farmer	76	59.92	4554.00
	Large scale farmer	44	61.50	2706.00
	Total	120		
Harvesting methods	Small scale farmer	76	60.50	4598.00
	Large scale farmer	44	60.50	2662.00
	Total	120		

**Table 9:** Mann-Whitney U test results according to land area

Awareness according to land area	Mann-Whitney U	Wilcoxon W	Z	P value
Land preparation	264.00	3190.00	-8.873	0.00
Sowing time	462.00	3388.00	-7.635	0.00
Sowing methods	462.00	3388.00	-7.635	0.00
Seed rate	1474.00	4400.00	-2.363	0.01
Seed treatment	1628.00	4554.00	-1.081	0.28
Seed varieties	1232.00	4158.00	-3.712	0.00
Irrigations	792.00	3718.00	-5.869	0.00
Intercultural practices	506.00	3432.00	-7.382	0.00
Fertilizations	176.00	3102.00	-9.492	0.00
Plant Protection	1496.00	4422.00	-2.218	0.02
Plant diseases	1474.00	4400.00	-2.363	0.01
Weedicide	1628.00	4554.00	-1.081	0.28
Harvesting methods	1672.00	2662.00	.000	1.00

### Comparison of Overall Awareness of Farmers According to their Land Area

Table 10 shows the comparison of overall awareness of farmers according to their land area; statistically significant difference was found with the P value=0.000. The large-scale farmers were aware of improved maize practices and small-scale farmers were unaware.

### Mean Rank of Awareness of Improved Maize Practices According to Visits to Extension Department

Awareness scores of farmers who visits agricultural extension department and who do not visit was compared

and Mann-Whitney U test was used to test the significant difference between two farmer's groups regarding visits to extension department. According to Table 11, farmers who visit extension department were aware about all improved maize cropping practices and other who were not visiting extension department were unaware. Only harvesting methods has equal mean rank.

### Mann-Whitney U Test Results of Awareness of Improved Maize Practices According Land Area

Table 12 demonstrated the difference between two farmer's groups was statistically significant at  $p < 0.001$ .

**Table 10:** Comparison of overall awareness of farmers according to their land area.

Groups	N	Mean Rank	Sum of Rank
Small scale farmer	76	55.00	4180.00
Large scale farmer	44	70.00	3080.00
Total	120		
Categories			Awareness
Mann-Whitney U			1254.00
Wilcoxon W			4180.00
Z			-3.60
P value			0.00

**Table 11:** Mann-Whitney U test with mean rank

	Do you visit agricultural extension department	N	Mean Rank	Sum of Ranks
Land preparation	no	42	32.50	1365.00
	yes	78	75.58	5895.00
	Total	120		
Sowing time	no	42	28.00	1176.00
	yes	78	78.00	6084.00
	Total	120		
Sowing methods	no	42	28.00	1176.00
	yes	78	78.00	6084.00
	Total	120		
Seed rate	no	42	52.14	2190.00
	yes	78	65.00	5070.00
	Total	120		
Seed treatment	no	42	58.64	2463.00
	yes	78	61.50	4797.00
	Total	120		
Seed varieties	No	42	43.36	1821.00
	Yes	78	69.73	5439.00
	Total	120		
Irrigations	no	42	23.36	981.00
	yes	78	80.50	6279.00
	Total	120		
Intercultural practices	no	42	27.00	1134.00
	yes	78	78.54	6126.00
	Total	120		
Fertilizations	no	42	34.50	1449.00
	yes	78	74.50	5811.00
	Total	120		
Plant protection	no	42	53.07	2229.00
	yes	78	64.50	5031.00
	Total	120		
Diseases	no	42	52.14	2190.00
	yes	78	65.00	5070.00
	Total	120		
Weedicides	no	42	58.64	2463.00
	yes	78	61.50	4797.00
	Total	120		
Harvesting methods	no	42	60.50	2541.00
	yes	78	60.50	4719.00
	Total	120		

Regarding the awareness of all improved maize practices showed significant differences among farmers who visited and not visited extension department excepted seed treatment, weedicide and harvesting methods.

#### Comparison of Overall Awareness of Farmers According to Visits to Extension Department

Table 13 shows the comparison of overall awareness of famers according to their visits to extension department, statistically significant difference was found with the P value=0.000. The farmers who visited extension department were aware of improved maize practices and other farmers who were not visiting extension department were unaware.

#### DISCUSSION

The current study was conducted to examine farmer's awareness of improved maize cropping practices in Punjab, Pakistan. Current study reveals dynamic nature of awareness regarding improved maize cropping practices and examine the variables including education, land area, visits to extension department and awareness. It explains differences between different farmers' groups regarding awareness.

Awareness of improved maize cropping practices among farmers is the initial step towards the understanding and adoption of improved cropping practice which effect on farmer's knowledge and socioeconomic status. The farmers who have more awareness are more likely to adopt

**Table 12:** Mann-Whitney U test results according to visits to extension department

Awareness according to visit to extension department	Mann-Whitney U	Wilcoxon W	Z	P value
Land preparation	462.00	1365.00	-7.488	0.00
Sowing time	273.00	1176.00	-8.702	0.00
Sowing methods	273.00	1176.00	-8.702	0.00
Seed rate	1287.00	2190.00	-4.233	0.00
Seed treatment	1560.00	2463.00	-1.935	0.05
Seed varieties	918.00	1821.00	-6.137	0.00
Irrigations	78.00	981.00	-10.512	0.00
Intercultural practices	231.00	1134.00	-9.000	0.00
Fertilizations	546.00	1449.00	-7.000	0.00
Plant Protection	1326.00	2229.00	-3.973	0.00
Plant diseases	1287.00	2190.00	-4.233	0.00
Weedicide	1560.00	2463.00	-1.935	0.05
Harvesting methods	1638.00	4719.00	.000	1.00

**Table 13:** Comparison of overall awareness according to visits to extension department

Groups	N	Mean Rank	Sum of Ranks
Yes	78	70.00	5460.00
No	42	42.86	1800.00
Total	120		
Categories			Awareness
Mann-Whitney U			897.00
Wilcoxon W			1800.00
Z			-6.448
P value			0.00

innovative agricultural technologies (Abeba & Belay, 2001; Rogers *et al.*, 2014). The results of our study found that (84.2%) of the farmers were aware of improved maize practices. Farmers might have high interest in improving their knowledge because of its high economic value in Pakistan (Go, 2016). Current results consistent with (Obayelu & Ajayi, 2018) which revealed that the majority of maize farmers were aware and farmers were trying to use improved maize seed varieties and organic fertilizer. Current result consistent with Onemolease & Okoedo-Okogie, (2007); Lawal *et al* (2004), that awareness of maize farmers regarding improved maize cropping technologies improved maize varieties.

Agricultural extension plays a role in enhancing farmers awareness as our results showed that the farmers who were used to visit extension department were aware of improved maize cropping practices, who were not used to visit to extension department were unaware. Current findings confirmed by Ajadi *et al* (2016), revealed that farmers who had contact with extension department were aware. These finding are also supported by Fawole & Tijani (2013) which showed high correlation between participation of farmers in extension activities and their awareness. According to (Umeh *et al* (2018), extension improved farmers' decision power, analytical abilities by communicating sound suggestions and information. Different extension approaches such as farmer field schools (FFS), training and visits (T&V) or participatory approach are used to share knowledge and skills with farmers (Maulu *et al.*, 2021). The past findings aligned with our results, contact with extension department increases farmers' awareness of improved maize practice in different ways, such as membership of farmer's association enhanced the awareness of existing and innovative farming technologies. Increasing in awareness of improved farm technologies increased the level of adoption (Chukwuji *et al.*, 2006). The contact with extension department and

participation in their field activities enhanced farmers' awareness of improved maize technologies as the previous research conducted by Mmbando & Baiyegunhi, (2016), found that farmers improved their awareness and adoption of alternative maize cropping technologies by the participation in extension events. The findings of the current study aligned with Obayelu & Ajayi (2018) which depicted that frequent extension contacts increased awareness among farmers and more likely increased the adoption. The frequent contact with extension department also increased awareness of improved maize technologies among farmers these results are similar to Ayinde *et al* (2010), reported that farmer's social and economic characteristics could impact on farmer's awareness of agricultural innovation. Like other studies it has been proved that high frequency of extension contacts improved the awareness of improved maize seed varieties (Sisay *et al.*, 2015).

Our findings revealed that large scale farmers were aware of improved maize cropping practices and small-scale farmers were unaware. Farmers who had large land area have high ability to accept and test improved cropping practices. Numerous studies are aligned with our results like Kamara *et al* (2018) confirmed that small scale maize farmers had less improved production awareness. It means large scale farmers have high information and awareness as compared to small farmers. Research conducted by Abbas *et al.* (2003) confirmed that large farmers in Pakistan have high awareness of improved sugarcane technologies (Abbas *et al.*, 2003). Large farmers might be more economically stable and spend money to get more information about improved maize cropping practices through advance channels. The results of current study are similar to Duhan & Dhingra (2018), revealed that the large farmers were aware of crop insurance because of their high income. Another result is showing similarity with our findings, large scale maize farmers have high awareness of climate change. Maize farmers who cultivated large land area were aware more conscious about their farm and impacts of climate change (Oduniyi, 2013).

Farmers who had been to college and higher education and large farmers were aware about improved maize cropping practices. The previous result is consistent with our findings, high formal education among farmers increased their knowledge and awareness of improved maize practices (Aturamu & Daramola, 2005). This result also aligned with Kadafur *et al* (2020), who stated that education level of maize farmers improved knowledge and

adoption of maize technologies. The core findings of Olila and Pambo (2014) confirmed our findings that education is one of the major determinants of awareness among farmers. Furthermore, Fisher *et al* (2019) had proved the role of education in awareness of improved maize practices, awareness among farmers increased by increasing education level. Another research also demonstrated that low education and small-scale farming have reverse effects on awareness and adoption, as previous results revealed that small-scale farmers were unaware of improved maize practices and low educational level were the reason behind low adoption of improved maize varieties. The farmer's awareness of new maize varieties depends on source of information such as easy access to extension could increase the adoption (Schroeder *et al.*, 2013). Another study confirmed our results that information seeking about improved practices increased adoption, access to extension department enhanced awareness of innovative agricultural technologies among farmers. Genius *et al* (2014) and Tijani and Nurudeen (2012), stated that large number of maize farmers were not aware of pesticide safety measure due to poor education and lack of awareness. Our results are also consistent with another study which depicted that highly educated farmers were aware of improve maize technologies than those who had low or no formal education (Okonji & Awolu, 2021). Education level of farmers increased abilities, awareness and adoption of innovative agricultural practice (Lavison, 2013).

## Conclusions

Current study provided insight into farmer's awareness of improved maize cropping technology. Results depicted that College or higher education, large land area and farmers' visits to extension department influenced on awareness of improved maize practices among maize farmers. The study provides useful implications for extension policy making regarding awareness of improved technologies. Extension programs should be implemented to update farmer's awareness of improved maize practices. On the basis of empirical findings, the study recommended that extension agents should fill existing awareness gap among farmers who have small land area, less educated and those who are not visiting extension department for innovative information of improved maize practices. It could be achieved by the application of various extension teaching methods, sound communication with farmers for example, Information and communication technology (ICT), Radio, television, agricultural journalism etc. This study was limited to Tehsil Jhang, province Punjab. The results may not be generalizable to maize growers who live in other provinces. Therefore, it is recommended that similar study should be carried out in other areas of Province.

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## Nutritional and Anti-nutritional Characteristics of Okra (*Abelmoschus esculents* (L.) Moench) Accessions Grown in Pawe District, Northwestern Ethiopia

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### ABSTRACT

Okra is one of the nutritious and multipurpose vegetables widely consumed in different parts of the world. The nutritional and other chemical profiles of accessions of the crop grown in the Pawe area, however, are not properly known. Therefore, the main objective of this study was to determine the nutritional and anti-nutritional content of thirteen indigenous okra accessions grown at Pawe District in Benishangul Gumuz regional state, Ethiopia. The result of the study showed that the nutritional composition has a significant ( $P \leq 0.05$ ) difference among okra pod accessions. The moisture, ash, protein, crude fat, crude fiber, and carbohydrate contents were ranged from 89.07 - 91.46, 7.16 - 8.25, 18.2 - 22.06, 0.88 - 1.43, 1.66 - 5.98, and 52.71 - 60.67 % respectively. While the content of oxalate, phytate, and tannin ranged from 0.56 - 0.73, 0.30 - 0.34, and 6.4 - 6.7 mg/100g, respectively, only oxalate content has significant ( $P \leq 0.05$ ) difference among accessions. Results indicate that okra pods are rich in nutrients and can contribute a vital role to human health. Further research is recommended on processing methods that influence the nutritional quality of okra pods.

**Key words:** Antinutrition, Okra, Proximate Composition, Indigenous Vegetable.

### INTRODUCTION

Worldwide, indigenous vegetables have long been part of traditional diets in communities. yet their nutritional value is unknown (Keatinge et al., 2014; Ojiewo et al., 2015). Especially, in Sub-Saharan Africa consistent with less information on their nutrition, the consumption level of vegetables is low. Therefore, understanding the nutritional value of indigenous vegetables is very important to solve food insecurity and malnutrition problems in developing countries (Kamga et al., 2013).

Okra [*Abelmoschus esculentus* (L.) Moench] is widely cultivated and utilized crops belonging to the Malvaceae family (Lamont, 1999; Sorapong, 2012). It is a widely cultivated and consumed nonleafy vegetable in tropical and subtropical areas (Aktaruzzaman et al., 2010; Gemedet et al., 2016). Different reports indicated that okra is originated in Ethiopia (Binalfew & Alemu, 2016; Sorapong, 2012) and then spread in to different countries of Middle East and North Africa (Lamont, 1999). The local name of okra is different from place to place. It is called lady's finger in England, gumbo in the United States of America, gombo in French, guibeiro in Portuguese, bamiah in Arabic, and

bhindi in India (Lamont, 1999; Singh et al., 2014). It is also named differently in Ethiopia as Kenkase (Berta), Andeha (Gumuz), and Bamia (Afan Oromo/ Amharic) (Gemedet et al., 2016).

Fresh and green seed immature okra pods are rich in protein dietary fiber, unsaturated fatty acid, minerals, and vitamins needed by humans and can be consumed as vegetables (Liu et al., 2021; Ndangui et al., 2010; Singh et al., 2014). Okra seeds contain 15-26% protein and more than 14% oil which is comparable with soybean. Its oil has high amounts of unsaturated fats such as oleic acid and linoleic acid, greenish-yellow color, pleasant taste and odor and therefore has vital role in human diet and can be used as an alternative source for edible oil (Kumar et al., 2010). Okra provides calcium, potassium, zinc, and other minerals which are often lacking in the diet of many developing countries (Hussein et al., 2018). Besides using as food, okra has several applications in pharmaceuticals and different industries (Adetuyi et al., 2011; Farooq et al., 2013; Kumar et al., 2013). Despite all these, less attention was given to improve its production (Kumar et al., 2010).

Characterizing variability in the overall quality and nutritional profiles of okra is important to clearly understand

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the added value to the common food items of the community in the locality and design alternative uses (Kamga et al., 2013). It is reported that the quality and functional properties of okra be affected by varieties, crop management, and processing methods (Hussein et al., 2018; Liu et al., 2021).

In Ethiopia, there is only little report on okra dealing with its genetic diversity (Binalfew and Alemu, 2016) and some nutritional composition (Gemedé, 2015; Zerihun et al., 2020). Hence, the aim of this experiment is to evaluate the nutritional and anti-nutritional characteristics of thirteen okra pod accessions grown in Pawe District, Benishangul-Gumuz Regional State, Northwestern Ethiopia.

## MATERIALS AND METHODS

### Sample Collection and Preparation

A total of thirteen okra accessions were collected from the research plots of Pawe Agricultural Research Center, Horticulture Research program. The collected okra pod samples were sliced manually to uniform size and thickness (5cm) using stainless- steel knife after washing it with distilled water (Gemedé, 2015) and were dried at 40°C in an oven for 24 hours. The samples were grounded until pass through 0.5 mm sieve by using an electric grinder. Finally, the samples were stored in refrigerator at 4°C packed with airtight plastic bag until analysis (Gemedé, 2015).

### Determination of Nutritional Composition

The proximate compositions; moisture content, crude protein, crude fat, crude fiber, and ash was determined by following the standard procedures of AOAC (2000). The moisture content was determined gravimetrically by drying the sample in an oven at 105°C for 24 hours till constant weight. Kjeldahl method was used to determine crude protein (%N\*6.25). Soxhlet extraction method was used to determine crude fat. The ash content was determined gravimetrically by incinerating the sample in a muffle furnace at 550°C for six hours. The carbohydrate amount was calculated by difference (Manzi et al., 2004). Crude fiber was determined by digesting the sample at a boiling point for 30 minutes with diluted (1.25%) sulphuric acid followed by 1.25% potassium hydroxide solution (AOAC, 2000).

### Determination of Anti-Nutritional Content

**Oxalate:** was determined by using the methods of the Association of Official Analytical Chemists (Anonymous, 2005). From a 100 ml conical flask containing one gram of sample, 75 ml of 3M sulphuric acid was added by string continuously in a magnetic stirrer for one hour. Then, the sample was filtered by using Whatman NO.42 filter paper. 25 ml of the filtrate was titrated with 0.1N potassium permanganate solution (KMnO<sub>4</sub>) at a hotplate (80 - 90 °c) until faint pink color appeared.

**Tannin:** The tannin content was determined calorimetrically following Joslyn (1970). Samples of 0.5 g of finely ground okra flour were defatted with five percent ethyl-ether for at least 15 minutes. Then, tannin was extracted using methanol and absorbance was measured at 760 nm.

**Phytate:** The phytate content was determined calorimetrically based on a method described by (Lapteva,

1988). From a 0.5g sample, 10 mL of 0.2 M HCl was added and incubated at room temperature for one hour and centrifuged for 30 minutes at 3000 rpm. After three milliliter of the supernatant was taken (excluding the blank sample), two milliliter of Wade reagent was added and homogenized and centrifuged the sample for 10 minutes at 3000 rpm. After the absorbance was measured using UV-Visible spectrophotometer, the phytic acid content was calculated from the absorbance versus concentration graph liner equation.

### Statistical Analysis

To compute the analysis of variance (ANOVA) SAS software version 9.4 was used. Duncan multiple range test (DMRT) was also employed to test the difference among treatments at 5% probability level.

## RESULTS AND DISCUSSION

### Proximate value

The proximate compositions of thirteen okra pod accessions are summarized in table 4.1. The result showed that the moisture content of fresh okra pod accessions varied from 89.07 - 91.46 % in wet base. Moisture content indicates the susceptibility of samples to microbial activity and is an index of water activity (Uyoh et al., 2013). The results were in agreement with other okra varieties in Nigeria having a moisture content ranged from 87.59 to 90.13% (Adetuyi et al., 2011) and matured Malaysian okra varieties (82.25%) (Nwachukwu *et al.*, 2014) . The ash content, which gives an idea of the mineral presence were ranged from 7.16% for P-08 to 8.25 % for M-10B and have significant difference ( $P \leq 0.05$ ) among accessions. The results were in line with other okra pod genotypes (Agbagoma and Balabi) with the ash content ranging 7.70 to 7.80% (Ofori et al., 2020).

The protein content ranges from 18.20% for M-12B to 22.06% for HUMERA and have significant difference ( $P \leq 0.05$ ) among okra accessions. This indicates that varieties have significant influences on the protein content. Oyelade et al. (2003) also reported that different varieties could have different protein content. Similarly (Adetuyi et al., 2011) showed that the protein content of different okra varieties significantly varies between 13.61 – 16.27%. Also, the result is in agreement with the findings reported by (Gemedé et al., 2016). This variability may be associated with the main effect of genetic factors (Adetuyi et al., 2011; Anwar et al., 2011). However, the okra pod accessions in this experiment have shown lower protein content as compared to some results such as Ofori et al. (2020) who reported protein content of 47.80 to 48.00%.

The result also indicates the crude fiber content also have significant differences ( $P \leq 0.05$ ) among accessions. The highest crude fiber (5.98) was obtained from HUMERA accession and the lowest (1.66%) is obtained from accession M-10B. The crude fiber of all okra pod accessions is somewhat lower than those reported by (Kouassi, 2013). The crude fat contents ranges from 0.88 to 1.43%. The highest fat content (1.43%) was recorded from accession M-27C, whereas the lowest (0.88%) were recorded from accession M-9A. These values are in line with the fat content (0.56 to 2.49 %) of okra genotypes reported by (Liu et al., 2021), but lower than the fat content

**Table 1:** Proximate Composition of Okra Pod Accessions.

Accession	Moisture	Ash	Protein	Crude fat	Crude fiber	Carbohydrate
	----- % -----					
HUMERA	89.53 <sup>ghi</sup>	7.50 <sup>bcd</sup>	22.06 <sup>a</sup>	1.28 <sup>b</sup>	5.98 <sup>a</sup>	52.71 <sup>h</sup>
M-10B	91.46 <sup>a</sup>	8.25 <sup>a</sup>	20.51 <sup>c</sup>	1.24 <sup>c</sup>	1.66 <sup>g</sup>	59.80 <sup>b</sup>
M-12B	89.07 <sup>j</sup>	7.25 <sup>cd</sup>	18.20 <sup>f</sup>	1.16 <sup>f</sup>	1.79 <sup>g</sup>	60.67 <sup>a</sup>
M-12E	89.98 <sup>de</sup>	7.60 <sup>bcd</sup>	19.55 <sup>d</sup>	1.23 <sup>cd</sup>	3.16 <sup>d</sup>	58.44 <sup>def</sup>
M-13A	89.80 <sup>ef</sup>	7.50 <sup>bcd</sup>	19.48 <sup>d</sup>	1.19 <sup>e</sup>	3.71 <sup>c</sup>	57.92 <sup>f</sup>
M-14A	89.38 <sup>i</sup>	7.50 <sup>bcd</sup>	19.48 <sup>d</sup>	1.15 <sup>fg</sup>	3.33 <sup>d</sup>	57.92 <sup>f</sup>
M-17A	89.43 <sup>hi</sup>	7.75 <sup>abc</sup>	20.46 <sup>c</sup>	1.22 <sup>d</sup>	1.78 <sup>g</sup>	58.22 <sup>ef</sup>
M-24B	90.01 <sup>d</sup>	7.67 <sup>bc</sup>	20.48 <sup>c</sup>	1.12 <sup>h</sup>	2.66 <sup>e</sup>	58.08 <sup>f</sup>
M-27C	91.15 <sup>b</sup>	7.84 <sup>ab</sup>	20.42 <sup>c</sup>	1.43 <sup>a</sup>	1.85 <sup>g</sup>	59.61 <sup>bc</sup>
M-30A	90.51 <sup>c</sup>	7.71 <sup>bc</sup>	19.75 <sup>d</sup>	0.91 <sup>j</sup>	3.22 <sup>d</sup>	58.92 <sup>cd</sup>
M-9A	89.66 <sup>fg</sup>	7.83 <sup>ab</sup>	21.13 <sup>b</sup>	0.88 <sup>k</sup>	4.35 <sup>b</sup>	55.47 <sup>g</sup>
P-08	89.60 <sup>fgh</sup>	7.16 <sup>d</sup>	20.22 <sup>c</sup>	1.14 <sup>g</sup>	2.27 <sup>f</sup>	58.81 <sup>de</sup>
P-2P1	90.55 <sup>c</sup>	7.85 <sup>ab</sup>	19.08 <sup>e</sup>	0.99 <sup>i</sup>	2.39 <sup>ef</sup>	60.25 <sup>ab</sup>
Mean	90.01	7.65	20.06	1.15	2.93	58.22
CV	0.14	3.91	1.09	0.80	7.62	0.71
Sig. ( $P < 0.05, 0.01$ )	**	*	**	**	**	**

Where: CV= coefficient of variation, Numbers in the same column followed by the same letter(s) are not significantly different at  $\alpha < 0.05$  (\*) or  $\alpha < 0.01$  (\*\*).

of Nigerian local varieties (9.03 to 10.57%) as indicated by (Adetuyi et al., 2011). The carbohydrate contents ranged from 52.71 to 60.67% and have a significance difference ( $P \leq 0.05$ ) among okra pod accessions. The highest carbohydrate content (60.67%) is obtained from accession M-12B and the lowest (52.71%) is obtained from accession HUMERA. The result is in the range of the carbohydrate content of two okra varieties (63.06 to 64.38 %) as reported by (Kouassi, 2013) and twenty *Tetrapleura tetraptera* pod accessions (51.17 to 66.29%) as reported by (Uyoh et al., 2013).

#### Anti-nutritional composition

The nutritive value and optimal utilization of foods are decreased by the chemical compounds synthesized naturally by normal metabolism of species known as anti-nutritional factors (Gemede et al., 2016). The antinutritional factors such as phytate, oxalate and tannin can form complexes with metallic ions such as Cd, Mg, Zn, and Fe and other minerals, thus decreasing their bioavailability (Mwanri et al., 2018).

The anti-nutritional (oxalate, phytate, and tannin) content of thirteen okra pod accessions are summarized in table 4.2. The result shows that the oxalate content ranged between 0.56 to 0.73 mg/100g and showed a significant difference ( $P \leq 0.05$ ) among accessions. The highest oxalate content (0.73 mg/100g) was obtained from accession M-10B, while the lowest (0.56 mg/100g) is obtained from accession HUMERA. The oxalate content of these accessions is in agreement with six Nigerian okra varieties having the oxalate content 0.32 to 0.51mg/100g as indicated by (Adetuyi et al., 2011). The phytate and tannin contents were ranged from 0.30 to 0.34mg/100g and 6.4 to 6.7mg/100g respectively. However, both the phytate and tannin content do not have significant ( $P \leq 0.05$ ) difference among accessions. The result is comparable with the antinutritional content of okra accessions reported by (Gemede et al., 2016).

#### Conclusion

This study indicates that the okra pods have a substantial amount of protein, dietary fiber, fat, and minerals and can be used as a good source of various nutrients needed by

humans. The okra pod accessions have high protein content ranging from 18.20 to 22.06%. Therefore, it could be a good protein supplement for other cereal-based foods. The result also suggested that there is wide variation in the proximate compositions among okra accessions. Therefore, breeders should also consider the nutritional and anti-nutritional profiles for their variety selections. However, further research is needed on the processing methods that influence the nutritional quality of okra pods.

**Table 2:** Anti-Nutritional Composition of Okra Pod Accessions

Accession	Oxalate (mg/100g)	Phytate (mg/100g)	Tannin (mg/100g)
HUMERA	0.56 <sup>e</sup>	0.30	6.4
M-10B	0.73 <sup>a</sup>	0.33	6.5
M-12B	0.71 <sup>ab</sup>	0.33	6.5
M-12E	0.72 <sup>ab</sup>	0.34	6.5
M-13A	0.68 <sup>abc</sup>	0.31	6.6
M-14A	0.63 <sup>cd</sup>	0.33	6.5
M-17A	0.66 <sup>bcd</sup>	0.31	6.6
M-24B	0.62 <sup>d</sup>	0.32	6.6
M-27C	0.73 <sup>a</sup>	0.32	6.7
M-30A	0.71 <sup>ab</sup>	0.33	6.6
M-9A	0.73 <sup>a</sup>	0.32	6.6
P-08	0.65 <sup>cd</sup>	0.33	6.7
P-2P1	0.72 <sup>ab</sup>	0.33	6.5
Mean	0.68	0.32	6.6
CV	5.09	4.67	2.62
Sig. ( $P < 0.05$ )	***	ns	Ns

Where: CV= coefficient of variation; Numbers in the same column followed by the same letter(s) are not significantly different at  $\alpha < 0.05$

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## Assessment of Agronomic Traits of Plantain Cultivars and Hybrids Cultivated at High-Density Planting

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### ABSTRACT

Plantain is one of the staple foods and source of income for the Ivorian populations. However, the cultivated varieties have low yields. Genotypes having diverse genetic background can improve the yield. This study was conducted in Anguédédou, in southern Côte d'Ivoire, to assess the agronomic performance of local cultivars and hybrids cultivated at high-density planting. Banana were planted in a randomized complete block design at a density of 2500 plants ha<sup>-1</sup> (2 m x 2 m). Six banana genotypes were tested during a production cycle. The results showed that hybrid SH 3640 had the shortest plant height (2.56 m) and harvest period of (11 months), the highest bunch mass (17 kg) and yield (16 t ha<sup>-1</sup>). The harvest period was longer with local varieties 3 Vert, Saci and Big Ebanga (around 15 months). The number of hands and fruit was higher in the hybrids. They had more than 8 hands and 90 and 73 fruits respectively for SH 3640 and BITA. Cultivar Big Ebanga produced the thickest (13 cm) and the longest (26 cm) fruit, while SH 3640 had the shortest fruit (18 cm). Hybrids and cultivars Saci, Big Ebanga and 3 Vert had the best yields and can be recommended to increase banana tree yield per hectare.

**Key words:** Growth, Yield, Agronomic Performance, Plantain, Hybrid, Côte d'Ivoire.

### INTRODUCTION

Formerly a household-farm crop, plantain has become over the years a crop with high added value. Its fruit is one of the main sources of food for the Ivorian populations. It is also a source of income for rural and urban populations, particularly women. They represent 80% of agricultural workers in plantain tree cultivation. Moreover, Côte d'Ivoire is the main supplier of the sub region. Despite its socio-economic importance, plantain yield remains low, around 4 t ha<sup>-1</sup> (Norgrove and Hauser, 2014 and FAOSTAT, 2022). In Côte d'Ivoire, plantain is grown in traditional systems with few inputs leading to a long-term loss of soil fertility because of the nutrients exported at each harvest and their intrinsic poverty. Farmers do not always have access to fertilizers, or either in a very limited way because of their high cost (FAO, 2016 and Guèdègbé and Doukkali, 2018) and availability. Furthermore, the cultivated varieties

are for the most part not very productive and susceptible to diseases and pests. Disease control strategies remain inaccessible to smallholder farmers with often limited resources (Traoré *et al.*, 2009). In order to improve plantain yield, hybrids and cultivars considered productive from various geographical areas have been introduced. These varieties, coupled with high-density planting (Khalequzzaman *et al.*, 2009 and N'guetta *et al.*, and Violet *et al.*, 2020 and Swain *et al.*, 2020), could increase plantain yield in Côte d'Ivoire. This study was therefore conducted to determine the yield potential of plantain cultivars and hybrids at high-density planting.

### MATERIALS AND METHODS

#### Area of Study

The experiment was conducted in southern Côte d'Ivoire, at the research station of Bimbresso on the site of

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Anguédédou. The geographical coordinates are 5°25' North latitude, 4°08' West longitude and 30 m altitude. The climate of the area, of the Attiean type, has two rainy seasons, the most abundant of which is centered on the months of May-June and the other on the months of October-November. The two rainy seasons are separated by two dry seasons. The average annual rainfall is around 2000 mm. The average temperature is 26°C, with minimum and maximum monthly values of 21°C and 32°C. This type of climate characterizes the dense forest. The soil is highly desaturated ferrallitic (CPCS classification) or Ferralsol drastic (WBR classification) from Tertiary sands. The texture is predominantly sandy-clayey, with a high proportion of coarse sand. The chemical characteristics of the soil determined in this study are given in Table 1.

### Plant Material

The plant material consisted of sword suckers of BITA 3 and SH 3640 hybrids and cultivars French Sombre, 3 Vert, Big Ebanga and Saci. The suckers taken from the collection of the National Center for Agronomic. The suckers were planted and monitored during a crop cycle.

### Experimental Design and Cropping Practice

The experimental design was a Fisher block with three repetitions for each variety. Each elementary plot consisted of 12 useful banana trees. A distance of 2 m was kept between banana trees and 2 m between rows, giving a total of 2500 plants ha<sup>-1</sup>. Six weeks after planting, the banana trees received dolomite and tricalcium phosphate at the rate of 165 g and 135 g, respectively. Urea and potassium chloride (KCl) fertilizers were added three months after planting. Each banana tree received 27 g of urea and 66 g of KCl for eight months. Weeding, leaf stripping and desuckering were carried out as needed. Weeding was manual for the first three months, then chemical.

### Data Collection

The behavior of the varieties with regard to black leaf streak disease through the youngest leaf affected (YLA) and the youngest necrotic leaf (YNL) was noted each month. But only data from two months before flowering (50% of shooting) of each variety were shown. At inflorescence emergence (flower stalk emergence), data was also collected on the banana trees. They focused on the height of the plant's collar up to the V formed by the last two leaves, the girth of banana trees at 1 m above ground, the number of emerged and living leaves. The latter was obtained by counting. A leaf is considered alive if 2/3 of its surface is green. The counting of the number of emerged leaves started three months after planting and took place every month until flowering. The number of days between planting and inflorescence emergence, from planting to harvest was calculated. At harvest, bunch mass, number of hands per bunch, number of fruits per bunch, fruit girth and length were taken. The yield was calculated taking into account the number of banana trees harvested and brought back per hectare.

### Statistical Analysis

The data collected was subjected to an analysis of variance using SPSS software. The means were separated by Tukey's test when the analysis was significant at 5% threshold.

## RESULTS

### Plant Height and Girth at Flowering

The highest pseudostem height (Table 2) was obtained with variety Big Ebanga (315 cm) followed by Saci (310 cm) and French Sombre (303 cm). Hybrid SH 3640 had the lowest height (256 cm) and differed statistically from other banana trees. Only varieties SH 3640 and 3 Vert had heights below 3 m. The girth of banana trees at flowering varied from 41 to 49 cm (Table 2). Cultivar French Sombre had the smallest girth and cultivar Big Ebanga the highest value.

### Number of Emerged and Functional Leaves at Flowering

Leaf emergence was different from one genotype to another. With an average of 44, more leaves emerged from cultivar Big Ebanga than the other banana trees (Table 3). Hybrids SH 3640 and BITA 3 had the lowest number of emerged leaves. These hybrids had respectively 35 and 39 emerged leaves and were significantly different ( $p=0.001$ ) from cultivars 3 Vert (42 leaves), Saci (42 leaves) and Big Ebanga (44 leaves).

The number of functional leaves at flowering was greater than eight in all banana trees (Table 3). Hybrid SH 3640 had the highest number of functional leaves (10) and was significantly different ( $p=0.012$ ) from cultivar Big Ebanga (8 leaves).

### Crop Duration of Banana Tree

The analysis of variance showed a significant effect ( $p=0.000$ ) between banana tree varieties for the crop duration characteristics (Table 4). Variety SH 3640 had the shortest cycle (days to shooting = 259 and days to Harvesting = 347). The crop cycle (days to harvesting) was long with varieties Saci (445 days), 3 Vert (445 days) and Big Ebanga (431 days). The flowers of these plantain trees appeared 11 months after planting (Table 4).

**Table 1:** Chemical characteristics of the soil of the study site in 0-30 cm depth

Parameters	Value
Water pH	4.4
C (gkg <sup>-1</sup> )	12.9
Nt (gkg <sup>-1</sup> )	1.4
Pass (mgkg <sup>-1</sup> )	43
MO (%)	2.21
CEC (cmol <sup>+</sup> kg <sup>-1</sup> )	7.2
Ca <sup>2+</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	0.522
Mg <sup>2+</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	0.397
K <sup>+</sup> (cmol <sup>+</sup> kg <sup>-1</sup> )	0.076

**Table 2:** Height and girth of the pseudostem of banana trees assessed at the shooting stage

Hybrids and Cultivars	Height (cm)	Girth (cm)
3 Vert	287 c	46.13 b
Saci	309.69 ab	48.13 ab
Big Ebanga	314.62 a	49.23 a
French Sombre	303.33 abc	41.53 c
BITA 3	295.59 bc	42.24 c
SH 3640	256.39 d	46.44 ab
Standard deviation	25.7	3.9
P-value	<0.0001	<0.0001

The means followed by the same letter in each column are not significantly different according to Tukey's test, at 5% probability.

### Bunch Characteristics Depending on the Varieties

The results presented in Table 5 show a variety effect on bunch mass ( $p < 0.0001$ ). All varieties except French Sombre had bunch masses greater than 12 kg. Bunches of hybrid SH 3640 had the highest mass with an average of 17.25 kg. Cultivar French Sombre produced the smallest bunches with a mass of 12 kg. The other cultivars had masses of about 14 kg ( $p = 0.98$ ).

The number of hands was around nine (9) with hybrids SH 3640 and BITA 3 (Table 5). In contrast, the cultivars had numbers of hands between 6 and 8.

A variety effect was also observed on the number of fruit per bunch ( $p < 0.0001$ ). Hybrids SH 3640 and BITA 3 gave more fruit per bunch compared to the cultivars (Table 5). Their numbers of fruit were 90 and 73, respectively. Cultivars Big Ebanga (48) and 3 Vert (43) had the lowest numbers of fruit per bunch.

The comparison of fruit girth showed a significant difference ( $p < 0.0001$ ) between the banana varieties tested (Table 5). Cultivar Big Ebanga had the largest fruits (13.36 cm). Fruit girth of hybrid SH 3640 (12.43 cm) did not differ statistically from cultivars 3 Vert (12.26 cm) and Saci (12.17 cm). French Sombre cultivar had the highest fruit girth value (11.16 cm). Contrary to the other bunch characteristics, the hybrids gave the shortest fruits (Table 5). Variety SH 3640 had the lowest fruit length value (18.53 cm) followed by BITA 3 (20.93 cm). Cultivar Big Ebanga gave the longest fruits (25.85 cm).

### Behavior of Varieties with Respect to Black Leaf Streak Disease

Differences were observed between varieties with respect to the YNL ( $p = 0.000$ ). Cultivar French Sombre with the lowest YNL value (7.19) differed from cultivars 3 Vert and Saci (Table 6). The latter two had a rank of necrotic leaves higher than 8. Hybrids BITA 3 and SH 3640 had YNLs statistically identical to cultivars 3 Vert, Saci, Big Ebanga. Regarding the YNL variable, there was no statistically significant difference (Table 6) between the varieties ( $p = 0.46$ ).

## DISCUSSION

### Agronomic Characteristics

Variety SH 3640 had a low habit (height less than 3 m), which gives it an advantage in areas with strong winds or steep slopes. This variety had the lowest number of emerged leaves, probably linked to its shorter production cycle which was 11 months. The number of functional leaves at flowering was greater than 8 leaves. This value

is the minimum number of leaves indicated by Lassoudière, (2007) for correct bunch development. The green leaves, by their photosynthetic capacity, allow a better filling of banana fruit and therefore acceptable yields.

In this study, the local varieties, except French Sombre, developed the inflorescence after 11 months far from the value indicated by Aubert, (1971). The latter reported that flowering generally occurs before 7 months after planting when the banana trees are in good growing condition. Banana tree cycle duration is influenced by several factors such as lack of water, planting density, plant material, temperature. Indeed, the more the planting density increases, the longer the crop duration lengthens (Biswas and Kumar, 2010 and Ndabamenye *et al.*, 2013). The increase in density to 2500 plants  $ha^{-1}$  could therefore be at the origin of the lengthening of the crop duration of genotypes up to 2 months longer compared to low density (CNRA, 2016). Lack of irrigation may also have contributed. The test was in rainy conditions. These results differ from those of Nankinga *et al.* (2005) in Uganda and N'guetta *et al.* (2015) in Côte d'Ivoire who observed no effect of plantain tree spacing (2 m x 2 m)

**Table 3:** Number of Emerged and Functional Leaves of Banana Trees Assessed at the Shooting Stage

Hybrids and cultivars	Number of emerged leaves	Number of functional leaves
3 Vert	41.69 b	8.5 ab
Saci	41.88 b	8.88 ab
Big Ebanga	44.23 a	8.11 b
French Sombre	40.47 bc	8.4 ab
BITA 3	38.82 c	9.07 ab
SH 3640	34.67 d	9.5 a
Standard deviation	3.6	1.3
P-value	<0.0001	0.005

The means followed by the same letter in each column are not significantly different according to Tukey's test, at 5% probability.

**Table 4:** Parameters of Crop Duration of the Bananas Assessed

Hybrids and cultivars	Days to shooting	Days to harvesting
3 Vert	358.48 a	444.73 a
Saci	360.8 a	445.67 a
Big Ebanga	347.25 a	430.63 a
French Sombre	298.62 b	385.14 b
BITA 3	288.81 b	374.14 b
SH 3640	259.33 c	346.89 c
Standard deviation	43	39.3
P-value	<0.0001	<0.0001

The means followed by the same letter in each column are not significantly different according to Tukey's test, at 5% probability.

**Table 5:** Banana Trees Yield Parameters

Hybrids and cultivars	Bunch mass (kg)	Number of hands per bunch	Number of fruit per bunch	Fruit girth (cm)	Fruit length (cm)	Yield ( $tha^{-1}$ )
3 Vert	13.64 cd	6.43 c	43.29 d	12.26 bc	23.13 b	12.28 cd
Saci	14.07 bc	7.6 b	58.4 c	12.17 bc	21.36 cd	12.81 bc
Big Ebanga	13.97 bc	6.85 bc	47.69 d	13.36 a	25.85 a	12.57 bc
French Sombre	12.14 d	6.68 c	58 c	11.16 d	22.46 bc	10.93 d
BITA 3	15.46 b	8.7 a	73.5 b	11.59 cd	20.93 d	13.91 b
SH 3640	17.25 a	8.8 a	90.27 a	12.43 b	18.53 e	16.04 a
Standard deviation	2.4	1.2	17.9	1.0	2.4	2.26
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

The means followed by the same letter in each column are not significantly different according to Tukey's test, at 5% probability.

**Table 6:** Phytopathological Parameters of Banana Trees

Hybrids and cultivars	YLA	YNL
3 Vert	3 a	8.5 a
Saci	3.25 a	8.75 a
Big Ebanga	3 a	8.06 ab
French Sombre	3.22 a	7.19 b
BITA 3	3.11 a	8.22 ab
SH 3640	2.85 a	7.65 ab
Standard deviation	0.7	1.4
P-value	0.46	0.0005

YLA: youngest leaf affected; YNL: youngest necrotic leaf. The means followed by the same letter in each column are not significantly different according to Tukey's test, at 5% probability.

in the first crop cycle. Hybrid SH 3640 was the most precocious of the varieties tested. It was harvested eleven months after planting before most cultivars started flowering. This result is different from the one obtained by Uazire *et al.* (2008) in Mozambique where SH 3640 banana trees were harvested after fifteen months, despite being planted at a lower density (3 m x 2 m).

Bunch mass and other yield components showed significant differences between varieties. Only hybrids had bunches of more than 15 kg, probably linked to their higher number of fruit and hands than that of local cultivars (Woomer *et al.*, 1999 and Cohan *et al.*, 2003 and Soares *et al.* 2012 and Dzomeku *et al.*, 2016). However, the yield of the cultivars was all above the national average (4 t ha<sup>-1</sup>). Fruit length of hybrid SH 3640 was less than 20 cm; which could constitute a handicap to its distribution. Consumers generally prefer long fruits. These two hybrids have, in a previous study in Côte d'Ivoire (Azaguié), given higher bunch masses (Kobenan *et al.*, 2009). This can be explained by higher rainfall and soil fertility in Azaguié.

### Phytopathological Parameters

The effect of leaf streak disease did not show any difference between hybrids and local cultivars two months after flowering. As the latter occurred in the dry season, the pressure of the pathogen was not strong due to the humidity which decreases during this period but also to the stripping carried out to limit the spread. Indeed, when humidity is high, it eases the development of the fungus<sup>21</sup>.

### Conclusion

All the banana trees tested exceeded the ideal size (3 m) except SH 3640 which had a low habit. Although the crop duration was long in all varieties, 11 to 15 months, the yields obtained were above the national average (4 t ha<sup>-1</sup>) by 63 to 75%. Hybrid SH 3640, which had the highest yield and the shortest crop duration, had the smallest fruits in terms of length. Cultivar French Sombre had the lowest yield.

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## Potential of Biogas and Organic Fertilizers Production through Anaerobic Digestion of Slaughterhouse Waste in Ouagadougou, Burkina Faso

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### ABSTRACT

The management of urban waste such as those from slaughterhouses is a major concern in developing countries. However, these wastes can be recycled as a source of energy and organic fertilizers for agricultural production. This study aimed to assess the possibility of producing biogas through anaerobic digestion of slaughterhouse wastes either digested sole or mixed with phosphate rock, and to characterize the chemical composition of the digestates obtained for agricultural production. Our results showed that the production of waste in the slaughterhouse of Ouagadougou, the main city of Burkina Faso is about 2.500 tonnes per year or about 200 tonnes per month. For slaughterhouse waste digested sole, the biogas production from the anaerobic digestion was in two months 139418 litres per ton of dry matter corresponding to a daily production of 2.2 litres per kg of dry matter. For slaughterhouse waste digested with phosphate rock, the biogas production was 120850 litres per ton of dry matter corresponding to a daily production of 1.9 litres per kg of dry matter. The digestates resulting from this digestion contained more than 50% of organic matter, 13 g/kg of nitrogen. Their pH was neutral (pH=7.18 for slaughterhouse waste digested sole, 7.47 for slaughterhouse waste digested with phosphate rock) compared to untreated waste which are acidic (pH 5.98). The carbon / nitrogen ratio of digestates was higher than 20. The adjunction of phosphate rock did not improve biogas production and the water soluble P content of the digestates. These results suggest that the anaerobic digestion of slaughterhouse waste could contribute to energy and organic fertilizers needs of populations while cleaning up the environment. However, there is a need to increase the duration of anaerobic digestion or to consider additional aerobic composting to further decrease the C/N ratio of digestates.

**Key words:** Anaerobic Digestion, Slaughterhouse Wastes, Phosphate Rock, Biogas, Organic Fertilizers, Ouagadougou.

### INTRODUCTION

Urban waste management is a major concern for municipalities in sub-Saharan Africa (Eaton and Hilhorst, 2003). Indeed, population growth and the rapid urbanisation of cities have resulted in a significant production of waste. This leads to a pollution of cities and the perpetuation of hygiene-related diseases (Kafando et al., 2013). Slaughterhouses are part of industries that produce a lot of waste that are not properly treated in many countries of sub Saharan Africa. However, these wastes are sources of organic matter and nutrients (Kiba et al., 2019) and can be recovered through anaerobic digestion for biogas and organic fertilizers production and then contribute to meet the growing energy and food demands of populations. Indeed, Kefalew et al. (2021)

estimated the potential of biogas production to 206.63 x 103 m<sup>3</sup>/year and biofertilizer production to 43184.9 kg of dry bio-fertilizer/year for a slaughterhouse in Ethiopia producing 688,755 kg per year. In Burkina Faso, studies in anaerobic digestion of some substrates such as manure have given interesting results. Indeed, the national bio-digester programme recorded in December 2011 a biogas production equivalent to 12080 bottles of butane gas of 12.5 kg corresponding to 382.54 tons of wood saved or 65.08 ha of preserved forest (Fasonet, 2012). The use of slaughterhouse waste, for example, in this bio-digester programme could then be very interesting in terms of its contribution to climate protection and sustainable soil management through the provision of organic fertilizers. In addition, digestion of slaughterhouse waste with rock phosphate could lead to organic fertilizers enriched in

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soluble phosphorus, which would contribute to improving the quality of the majority of soils in sub-Saharan Africa that are known to be phosphorus deficient.. This study aimed: (1) to estimate the slaughterhouse waste production in Ouagadougou in Burkina Faso (2) to evaluate the quantity of biogas produced during the anaerobic digestion of the slaughterhouse waste sole or combined with rock phosphate and (3) characterize the chemical composition of the digestate produced after anaerobic digestion of this waste for agronomic purposes.

## MATERIALS AND METHODS

### Materials

The studied waste was collected from the main slaughterhouse of Ouagadougou, the main city of Burkina Faso. This waste is essentially composed of the contents of stomachs and intestines from the slaughter of cattle, small ruminants, pigs and sometimes camels. The selected chemical parameters of this waste are shown in the Table 1.

### Assessment of waste production

The quantities of waste produced by the slaughterhouse of Ouagadougou were estimated from the number of animals slaughtered and the average weight of the contents of these animals' bellies. Thus the average weight of the contents of the bellies was evaluated at 33.43 kg per animal for large animals such as cattle and at 2.48 kg and 2.69 kg respectively for small ruminants and pigs.

### Anaerobic digestion and measurements of released gas

The anaerobic digestion was carried out according to the method described in Sedego et al. (1989) based on the use of pits installed at the Institute of Environment and Agricultural Research station of Saria in Burkina Faso (Picture 1). A quantity of 1.2 tons of waste was used to fill each tank. For the digestion of waste with phosphate rock, 36 kg of phosphate rock was added and mixed thoroughly. The waste was moistened to saturation to create anaerobic conditions. The water supply was staggered during the

aerobic pre-fermentation phase to ensure saturation of the waste. The pre-fermentation lasted 2 days and the tanks were closed until the end of the digestion process. The biogas produced was measured daily with a gas meter. The duration of anaerobic digestion was 63 days.

### Evaluation of selected chemical parameters of the composts produced

The pH of was determined by a pH meter (WTW InoLab, Weilheim, Germany) according to Walinga et al., (1989) with a waste/water ratio of 1/5. The organic matter (OM) content of the waste was determined by calcination in a CARBOLITE muffle furnace at 550°C for 2 hours. The organic matter was calculated by the following formula:

$$OM (\%) = \frac{(W_i - W_f)}{W_i} \times 100$$

$W_i$  is the initial weight of the test sample and  $W_f$  is the final weight of the test sample after calcination.



**Picture 1:** A pit installed at the agricultural research station of Saria, Burkina Faso for anaerobic digestion.

**Table 1:** Selected chemical parameters of slaughterhouse waste collected from the main slaughterhouse of Ouagadougou, Burkina Faso

Parameters	O M (%)	Total N (g/kg)	C/N	Total P (mg/kg)	Water soluble P (mg/kg)	pH water
Values	88.4	18.1	28	2086	648	5.98

**Table 2:** Average quantity in tons of solid waste produced in the main slaughterhouse of Ouagadougou, Burkina Faso

Month	2005	2006	2007	2008
January	221.69	208.75	142.55	200.14
February	212.33	200.99	133.95	168.60
March	269.02	206.16	189.45	176.31
April	265.41	207.95	197.99	180.53
May	232.25	222.32	212.76	206.76
June	255.82	208.84	197.10	174.25
Jully	240.76	196.38	199.68	174.44
August	235.37	190.61	187.40	160.85
September	226.60	187.63	190.36	172.01
October	244.23	184.46	224.06	174.14
November	253.32	176.04	227.41	186.82
December	287.69	185.70	231.62	189.81
Annual production	2944.50	2375.83	2334.35	2164.66
Average of four years	2454.84±339			

For the determination of total nitrogen and total phosphorus content, waste samples were first digested with concentrated sulfuric acid in the presence of hydrogen peroxide, selenium and salicylic acid. Phosphorus and nitrogen in the resulting solutions were measured using an automatic colorimeter SKALAR (Skalar SAN plus Segmented flow analyzer, Model 4000-02, Breda, Holland) (Okalebo et al. 2002). The extraction of water soluble phosphorus was done according to the method described in Lompo (1993).

## RESULTS

### Production of solid waste at the slaughterhouse of Ouagadougou

The monthly production of waste in the slaughterhouse of Ouagadougou is shown in Table 2. The average waste production was estimated to about 2.500 tonnes per year, corresponding to about 200 tonnes per month.

### Biogas production during the anaerobic digestion of slaughterhouse waste (SW)

The biogas production during anaerobic digestion is presented in Figure 1. A significant biogas production was recorded with SW, which started from the first day after the closure of the tanks. After 63 days of anaerobic digestion, for SW digested sole, the total amount of biogas produced was 139418 litres per ton of dry matter, i.e. an average daily production of 2.2 litres per kg of dry matter. SW digested with phosphate rock produced lower quantity of biogas compared to SW composted sole, with a total amount of biogas of 120850 litres per tonne of dry matter, i.e. an average daily production of 1.9 litres per kg of dry matter.



**Fig. 1:** Cumulative biogas production during anaerobic digestion of slaughterhouse waste and slaughterhouse waste combined with phosphate rock.

### Selected chemical parameters of the digestates

The results in Table 3 show the chemical characteristics of the digestates of slaughterhouse waste after anaerobic digestion. We noted that the composts resulting from the anaerobic digestion of this waste are rich in organic matter, which represents more than 50% of the dry weight of this waste. They are sources of phosphorus and nitrogen. Compared to raw slaughterhouse waste, which has an acidic pH of 5.98, we note that the digestates resulting from the anaerobic digestion have a neutral pH. These digestates also have lower nitrogen contents compared to raw slaughterhouse waste. The digestion of sole slaughterhouse reduced their ratio C/N, however this ratio remained higher than 20. The digestion of slaughterhouse waste with phosphate rock did not reduce their ratio C/N. Slaughterhouse waste digested with phosphate rock content higher total P but lower water soluble P compared to raw slaughterhouse waste and slaughterhouse digested sole.

## DISCUSSION

Slaughterhouse wastes are a potential source of recoverable organic matter. The analysis of the waste production at the main slaughterhouse of Ouagadougou in Burkina Faso showed that this resource is regularly produced and available a whole year. Considering the various other slaughterhouses throughout the country, we can conclude that slaughterhouse waste can be considered as a potential source of recoverable organic matter and energy production in the country.

The production of biogas during the digestion process is the result of the decomposition of the organic matter of the different substrates by anaerobic microorganisms (vögeli et al., 2014; Náthia-Neves et al., 2018). The average daily production of biogas of 2.2 l/kg dry matter and 1.9 l/kg dry matter respectively for slaughterhouse waste digested sole and slaughterhouse waste digested with phosphate rock are higher than those obtained by Lompo (1983) and Sedogo et al. (1989) with a mixture of sorghum straw and cow dung (1.28 litres per kg dry matter and 1.6 litres per kg dry matter respectively). These high biogas production are probably related to characteristics of this slaughterhouse waste, namely their high organic matter content (88,4%) and their C/N ratio favourable for anaerobic digestion. Despite that the optimum range of C/N ratio favourable for anaerobic digestion was reported to be between 20 and 25, some authors (Yong et al., 2015; Dadaser-Celik et al., 2016) indeed noted optimum biogas production using substrates with C/N ratio of 28 and 31. The studied slaughterhouse waste from the city of Ouagadougou with a C/N ratio of about 28 is therefore a potential source of raw material for biogas production that could partially offset the high cost of energy.

**Table 3:** Selected chemical parameters of digestates from an anaerobic digestion of slaughterhouse waste (SW) and slaughterhouse waste combined with phosphate rock (PR).

Treatments	MO (%)	N g/kg)	C/N	Pt(mg/kg)	Water soluble P(mg/kg)	pHwater
Sole SW digestate	55.1	13.0	25	2085.9	546.5	7.18
SW digested with PR	68.51	13.0	31	9579.43	377.2	7.47

Digestates of slaughterhouse waste exhibited higher pH compared to raw slaughterhouse waste. The increase of substrate pH after anaerobic digestion was reported by several authors and was partially linked to the removal of carbonic gas and the formation of ammonium carbonate during digestion (Möller and Müller, 2012; Owamah et al., 2014). The less acidic pH of the composts may be of interest for soils in Burkina Faso that become acidified after cultivation without a proper management. The C/N ratio of digestates remained higher than 20 after 63 days of digestion suggesting that the digestate are not well decomposed (Mustin, 1987). This shows a need to increase the duration of anaerobic digestion or to consider additional aerobic composting to further decrease the ratio C/N of the digestates. Indeed, a high C/N ratio of organic fertilizers could lead to a nitrogen deficiency and therefore a decrease in crop production. The digestates from anaerobic digestion have lower nitrogen contents than raw slaughterhouse waste. These results corroborate with those of Aklaku et al (2006) who noted a decrease in nitrate contents after the anaerobic digestion of slaughterhouse waste. The addition of phosphate rock during the digestion led to an increase of the total P content of the digestates while the increase of the pH during digestion process (table 2) led to the lower water soluble P content of the digestates compared to raw slaughterhouse waste. Indeed, in alkaline condition the adsorption of soluble P by cations such as calcium occurs (Johan et al. 2021).

## Conclusion

Slaughterhouse waste is a suitable source of substrates for biogas production by anaerobic digestion. They are regularly produced in the cities of Ouagadougou and have an average daily biogas production of 2.3 litres per kg of dry matter and 1.9 litres per kg of dry matter respectively for slaughterhouse waste digested sole and slaughterhouse waste digested with phosphate rock. The biogas production from slaughterhouse waste will allow diversifying energy sources and to provide a low cost energy source for the populations. The compost resulting from the production of biogas by anaerobic digestion is a source of organic fertilizers with pH near neutrality and rich in nutrients such as nitrogen and phosphorus that can improve agricultural production. However, the high C/N ratio of the digestates suggests a need to increase the duration of anaerobic digestion or to consider additional aerobic composting for decreasing the C/N ratio. The adjunction of phosphate rock does not improve the biogas production and the water soluble P content of the digestates.

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## Cloning and Characterization of Trichoderma Glucanase Gene for Plant Transformation

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### ABSTRACT

*Trichoderma harzianum* is a potential biocontrol agent. A number of mechanisms showing their mycoparasitic ability have been explored i.e., antibiotic production, production of hydrolytic enzymes, parasitism, competition for nutrients, defense mechanism stimulations, and combination of all these. Considering the importance of *Trichoderma harzianum* derived glucanases in mycoparasitism, the present research work was planned to identify, isolate and characterize pathogenesis-related  $\beta$ -1, 6-glucanases gene from local strain of mycoparasite for plant transformation. *Trichoderma harzianum* genomic DNA was isolated by using modified CTAB method. Particular restriction sites were engineered at the far ends of  $\beta$ -1, 6-glucanase gene by Polymerase Chain Reaction. The amplified glucanase gene with engineered sites was cloned into pTZ57R/T cloning vector. Cloned fragment was sequenced and the resultant sequence was analyzed by using various bioinformatics tools i.e., Blast, Restriction mapper. It was found to have more than 97% homology with glucanase sequences reported in literature. Then regulatory sequences were cloned at the far ends of characterized glucanase gene for the development of plant transformation vector.

**Key words:** Glucanase Gene, Plant Protection, Bioinformatics

### INTRODUCTION

Agriculture production affected by fungal diseases. Antifungal genes have been inserted into plants to developed transgenic plants that confer resistance against the pathogens (fungal pathogens). Transgenic plants that were resistance to fungal have been developed by inserting (cell wall degrading enzymes) genes that degrade fungal cell wall for example chitinase and glucanase (Ceasar *et al.*, 2012). Considerable yield losses had been observed in in large number of crops due to fungal diseases. Production of enzymes (cell wall degrading enzymes) play vital role in defense response of plants because these enzymes were able to degrade the cell wall of attacking plants pathogenic fungi. In transgenic plants (fungal resistance plants) this natural mechanism of defense was improved. Disease resistance have been improved genetically by plants breeding techniques but this is valid only within species

that were sexually compatible and can take up to 15 to 20 years (Rommens and Kishore 2000). Recognition of biological agents in combination with molecular biology is a significant alternative to control plant diseases. Microorganisms having antifungal/antimicrobial properties are probable source of genes to give disease resistance in plants. Biocontrol agents that can be used for disease management are fungi, bacteria and actinomycetes etc. Among the various biocontrol agents like *Bacillus subtilis*, *Penicillium spp.*, *Pseudomonas fluorescence*, *P.putida*, *Glomus mossae*, *Gliocladium* etc., *Trichoderma* spp. have proved more efficient to intricate most of the phytopathogens. The means of action of *Trichoderma* is suggested to be either by competition for food, shelter, mycoparasitism, antibiosis or a combination of all of these (Elad, 1996).

The genome sequencing of *Trichoderma reesei* revealed that it is comprised of fewest of genes that encodes

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for cellulolytic and hemicellulolytic enzymes (Martinez *et al.*, 2008). So genetic engineering has become a useful alternative. Valuable traits are being introduced into model organisms by the facility of molecular techniques. Transformation of genetically determined characters eliminates the species boundaries that have conventionally restricted germplasm sources. The advancement in Molecular Biotechnology have enabled scientists to identify, isolate and characterize plant stress responsive genes for plant transformation elaborating their role to combat stresses (Azevedo *et al.*, 2011).

The aim of this study is to prepare the expression vector for plant transformation. For this, *Trichoderma harzianum* genomic DNA was isolated by PCR. The amplified glucanase gene with engineered sites was cloned into pTZ57R/T cloning vector. Cloned fragment was sequenced and the resultant sequence was analyzed by using various Bioinformatics tools i.e., Blast, Restriction mapper. It was found to have more than 97% homology with glucanase sequences reported in literature. Then regulatory sequences were cloned at the far ends of characterized glucanase gene for the development of plant transformation vector.

## MATERIALS AND METHODS

### Growth media for *Trichoderma harzianum*

#### Preparation of Potato Starch

Potato dextrose is a widely used growth media for different fungal pathogens and is abbreviated as PDA. Potato starch was prepared for PDA by boiling 500 g of potato (washed and peeled) in 1000 ml of distilled deionized water until volume remained half. Potato infusions were decanted through muslin cloth and potato starch was autoclaved at 121°C for 15 minutes at 15 psi pressure and stored at 4°C.

### Maintenance of *Trichoderma harzianum* Cultures

*Trichoderma Harzianum* was maintained on potato dextrose agar (PDA). Sterilized medium was poured in 10 ml test tubes. These test tubes were allowed to solidify at slightly angled position to prepare slants. The PDA slants provides an array of surfaces that are exposed to oxygen containing air in varying degrees. These slants were incubated with fungal spores by using inoculating loop in laminar air flow cabinet and placed in incubator at 28°C for 72 hours in dark.

### Isolation of Total Cellular DNA from *Trichoderma harzianum*

Genomic DNA was isolated by using protocol described by Plattner *et al.* (2009) with certain modifications. CTAB method was used to isolate the genomic DNA. Quality of genomic DNA was checked by gel electrophoresis.

### Amplification of Glucanase Genes from *Trichoderma harzianum*

For glucanase gene isolation from *Trichoderma Harzianum*, specific primers were used. Sequences of forward primer: 5'GC CTCGAGCCATCAAGATGAAGTACTC 3' reverse primer: 5' GCACGCGTCAATCACTCGTGATTACC 3.

Total volumes of 25µl PCR reaction was prepared for each PCR tube in which 0.25µl *Taq polymerase*, 0.25µl forward and 0.25µl reverse primer, 1.5µl MgCl<sub>2</sub>, 2.5µl *Taq* buffer, 1.5µl dNTPs and different volumes of genomic DNA was used. PCR profile was as followed, PCR amplification was done incubating the PCR tubes at 95 °C for 5 minutes. Then 35 cycles comprising of denaturation at 95 °C for 1 minute and 30 seconds, annealing of primers at 51.3 °C for 1 minute and 30 seconds and extension at 72 °C for 2 minutes was carried out. Then final extension was carried out at 72 °C for 10 minutes. And finally, PCR product was run on 0.8% gel electrophoresis. Amplicon product was eluted from the gel and ligation, 14.5 µl PCR product, 1 µl vector (pTZ57R/T), 4 µl buffer, 0.5 µl T4 DNA ligase enzyme was added into PCR tube. Mixed it gently and incubated it in incubator for 16 hours at 16 °C. After the incubation of 16 hours.

### Transformation and Confirmation

LB agar plates were streaked with Top-10 strain and incubated it at 37°C for 16 hours to get colonies. The 5 µl ligation product was added into the cells by the help of pipette. The cells were kept on ice for 10 minutes then heat shock was given by the heat block at 42°C for 90 seconds and again on ice for 5 minutes. 800 µl LB liquid was added in the competent cells and gently shake. Kept eppendorf tube of competent cells on shaker at 37°C for 1.5 hours. After incubation, centrifugation was done for 3 minutes at 5000rpm. Discarded 900 µl supernatant and in remaining 100 µl dissolve the pellet. Overnight incubation of plates at 37 °C, blue and white colonies appeared on the plate. Blue colonies were transformants harboring circular vector only with intact lac-Z gene which encodes for β-galactosidase that stimulated by IPTG and use X-gal as substrate to give a blue colored product. White colonies are recombinants harboring vector with ligated insert at MCS (multiple cloning sites). This MCS is present in Lac-Z gene and this insertion disrupts Lac-Z gene and hence gives white colonies. With a sterile tooth pick, single white colonies were picked and shifted to LB fortified with antibiotic, and incubated it at 37 °C in shaking incubator for 16 hrs. The plasmid DNA was isolated using alkaline lysis procedure illustrated by Birnboim and Doly (1979). For plasmid isolation FavorPrep™ Plasmid DNA Extraction Mini Kit was used for plasmid extraction. Quality of plasmid was checked on 0.8% agarose gel.

### Confirmation of DNA through Restriction of Plasmid

Eluted plasmids were digested with restriction endonucleases and 20 µl was total volume of reaction mixture having 10X reaction Tango buffer (Fermentas, USA or Invitrogen, USA). The amplicon was cut from the plasmid for size confirmation with *EcoRI* and *HindIII*. For the visualization of the restricted fragments 0.8% agarose gel was used and restricted fragments were visualized under UV light. Liquid LB culture containing transformed cells with cloned fragment were mixed with 100% glycerol in 1:1 ratio. Labelled it and stored it at -80 °C for future use.

### Sequencing and Bioinformatics Analysis

BLAST (Basic Local Alignment Search Tool) was used to find homology of the target sequence with the sequences present in database. Phylogenetic tree was

constructed using BLAST. ExPASy (the Expert Protein Analysis System) translate tool was used to translate nucleotide sequence into amino acid sequence. Phyre<sup>2</sup> (Protein Homology/Analogy Recognition Engine) was used to predict structure of protein.

### Cloning of Glucanase Gene in Expression Vector pLH7000M

#### Restriction of T/A Cloned Vector

Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *XhoI* to cut plasmid at restriction sites present in pLH7000M expression vector and the digested product was resolved on 0.8% agarose gel. It resulted in desired DNA fragments of 2700 bps (vector backbone) and 1500 bps (insert). Amplicon product was eluted from the gel and ligation. Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *XhoI* to cut plasmid at restriction sites present in pLH7000M expression vector and the digested product was resolved on 0.8% agarose gel. It resulted in desired DNA fragments of 8500 bps and 411 bps restricted fragment of glucanase from T/A clone and pLH7000 was ligated. For ligation, 14.5 µl PCR product, 1 µl vector (pLH7000M0), 4 µl buffer, 0.5 µl T4 DNA ligase enzyme were added into PCR tube. Mixed it gently and incubated it in incubator for 16 hours at 16 °C. After the incubation of 16 hours, it is stored at 4 °C.

#### Bacterial Transformation

LB agar plates were streaked with Top-10 strain and incubated it at 37°C for 16 hours to get colonies. The 5 µl ligation product was added into the cells by the help of peptide. The cells were kept on ice for 10 minutes then heat shock was given by the heat block at 42°C for 90 seconds and again on ice for 5 minutes. Added 800 µl LB liquid in the competent cells and gently shake. Kept eppendorf tube of competent cells on shaker at 37°C for 1.5 hours. After incubation centrifugation for 3 minutes at 5000rpm. Discarded 900 µl supernatant and in remaining 100 µl dissolve the pellet. Overnight incubation of plates at 37 °C, blue and white colonies appeared on the plate. Blue colonies were transformants harboring circular vector only

with intact lac-Z gene. With a sterile tooth pick, single white colonies were picked and shifted to LB fortified with antibiotic, and incubated it at 37 °C in shaking incubator for 16 hrs. The plasmid DNA was isolated using alkaline lysis procedure illustrated by Birnboim and Doly (1979). Eluted plasmids were digested with restriction endonucleases and 20 µl was total volume of reaction mixture having 10X reaction Tango buffer (Fermentas, USA or Invitrogen, USA). The restriction mixture was incubated at 37 °C for overnight. The enzymes used for plasmid DNA linearization and for confirmation of plasmid size were *MluI* and *XhoI*. The amplicon was cut from the plasmid for size confirmation with *MluI* and *XhoI*. For the visualization of the restricted fragments 0.8% agarose gel was used and restricted fragments were visualized under UV light. Liquid LB culture containing transformed cells with cloned fragment were mixed with 100% glycerol in 1:1 ratio. Labelled it and stored it at -80 °C for future use.

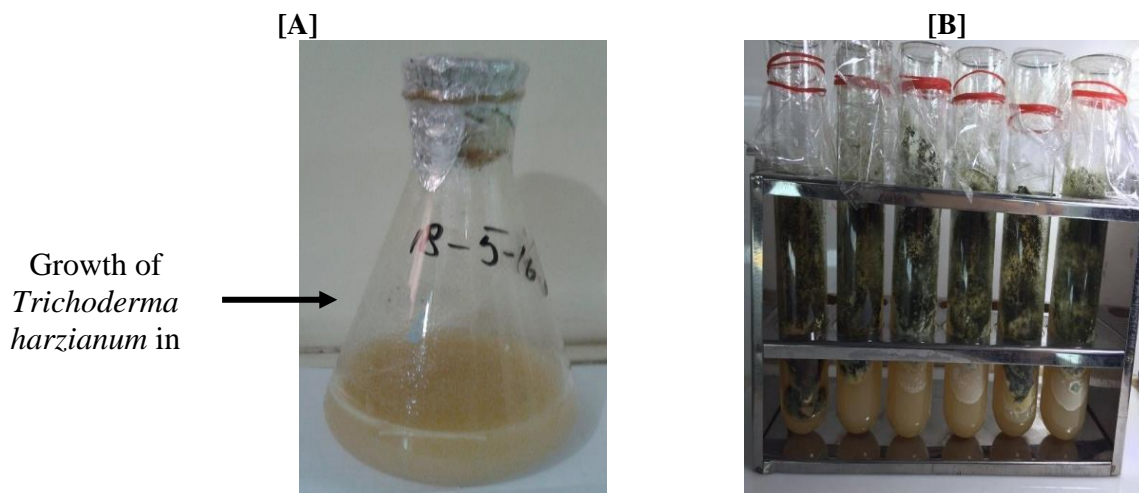
#### Sequencing and Analysis

BLAST (Basic Local Alignment Search Tool) was used to find homology of the target sequence with the sequences present in database. Phylogenetic tree was constructed using BLAST. ExPASy (the Expert Protein Analysis System) translate tool was used to translate nucleotide sequence into amino acid sequence. Phyre<sup>2</sup> (Protein Homology/Analogy Recognition Engine) was used to predict structure of protein.

## RESULTS

### Optimization of Growth Conditions for *Trichoderma harzianum*

*Trichoderma* strain was obtained from department of Plant Pathology, University of Agriculture Faisalabad and was cultured in Potato Dextrose Broth (PDB) (Figure 1A), and Potato Dextrose Agar (PDA) which is common growth media for fungus. The fungus was grown in Erlenmeyer flask containing PDB and glucose was used as source of carbon. *Trichoderma Harzianum* was also cultured in test tubes containing PDA media Figure 1 B, and was placed in dark at 28°C.



**Fig: 1.** Growth of *Trichoderma harzianum* in Potato Dextrose Broth and Potato Dextrose Agar. A) Green colored mycelium of *Trichoderma Harzianum* grown in PDB B) *Trichoderma Harzianum* grown on PDA slants.

### Culturing of *Trichoderma Harzianum* in Potato Dextrose Agar Media in Petri Plates

*Trichoderma harzianum* was cultured in Potato Dextrose Agar Media with chloramphenicol antibiotic as selective agent. After two to three weeks at 28°C in dark, *Trichoderma harzianum* growth was observed on petri plates Fig 2.

### Amplification of Glucanase Gene to Engineer Particular Restriction Sites

Glucanase gene was amplified using gene specific primers. Nucleotide sequence specific to *MluI* restriction endonuclease was introduced at the 5'-end of gene whereas

*XhoI* specific site was introduced at 3'-end of glucanase gene. Various parameters were optimized for the amplification of gene including concentration of template DNA, annealing temperature of primers and  $MgCl_2$  concentration etc. These primers resulted in best amplification at 52.1°C whereas size amplicon was 1330 bps as per expectations Figure 3.

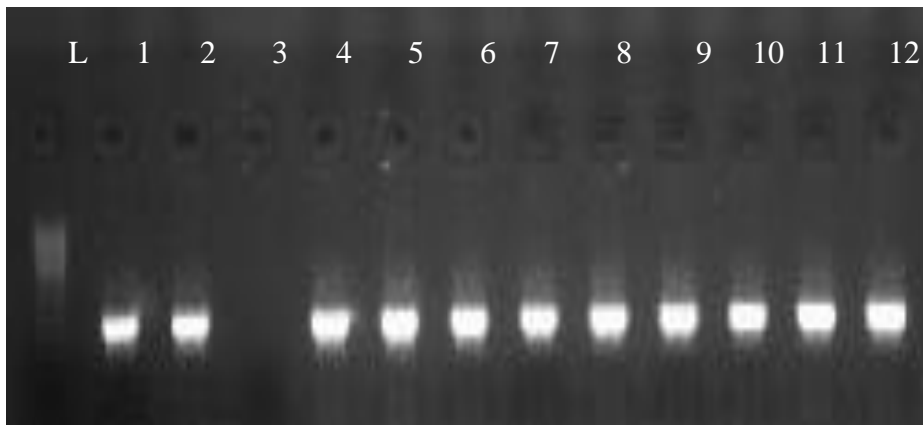
### Cloning of Glucanase Gene

The amplified PCR product was cloned into a vector (T/A cloning vector) for the characterization of Glucanase gene Fig 4.

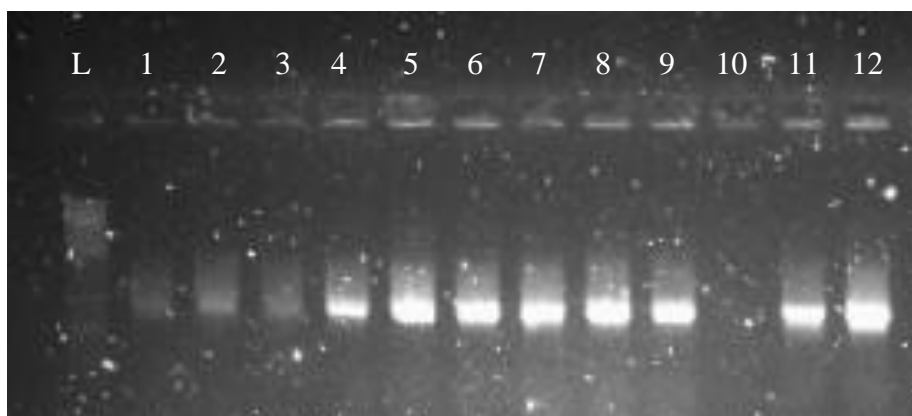
*Trichoderma  
Harzianum*  
Grown on PDA  
slants



**Fig: 2.** Growth of *Trichoderma harzianum* in Potato Dextrose Agar Media.



**Fig: 3.** Amplification of Glucanase gene using gene specific primers to introduce specific sites of *MluI* and *XhoI*.



**Fig: 4.** Amplification of glucanase gene from *Trichoderma Harzianum*. The best results were obtained at a melting temperature of 52.1°C.

### Elution and Ligation of PCR Product

The desired amplified DNA fragment was eluted from the gel or precipitated and was ligated into pTZ57R/T vector. 100 µl PCR product was precipitated using 15 µl sodium acetate and 2/3 volumes of chilled isopropanol. Then it was incubated at -20°C for 30 minutes and was centrifuged at 13,200 rpm speed for 12 minutes. Then supernatant was discarded & pellet was air dried so that all of the isopropanol was evaporated. Then air dried pellet was re-suspended in 20 µl of double distilled deionized autoclave water and was run on gel to see concentration of the eluted/precipitated product. The ligation mixture was then incubated at 16°C for 16 hours followed by transformation in the *E.coli* bacterial cells or stored at -20°C.

TA cloning is a one-step direct cloning of the PCR products and generated 3'-dA overhangs via *Taq* DNA polymerase or other thermostable DNA polymerases. *Taq* DNA polymerase lack proof reading ability. TA cloning vector is linearized pTZ57R/T plasmid (Figure 5) with

Eco321 and have a tail of single ddT. This 3'-ddT overhangs prevent the re-circularization of vector because this 3'-ddT overhangs present at both ends of the vector during ligation, resulting in high cloning yields and low background.

### Transformation and Selection of Transformed Bacterial Cells

Competent cells of Top 10 strain of *Escherichia coli* were transformed with ligation mixture by heat shock method. Transformed cells (25 µl, 50 µl and 100 µl) were spread out on LB plates fortified with 100 mg/L ampicillin. For blue-white selection of bacterial colonies, IPTG (100mg/L) and X-gal (100 mg/L) were also spread out on LB plates along with transformed bacterial cells, with the help of a sterilized glass spreader. These plates were incubated in an incubator at 37 °C for 16 hours. After incubation of 16 hours, blue white-colonies appeared on the plates as shown in Fig 6.

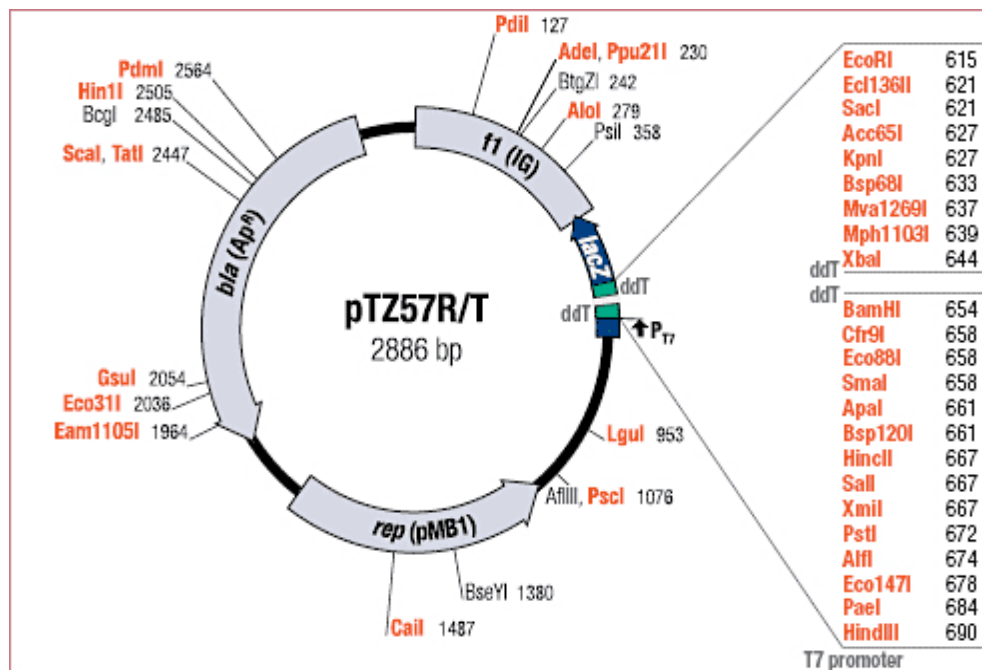


Fig 5. Map of pTZ57R/T cloning vector showing multiple cloning sites and selection markers.

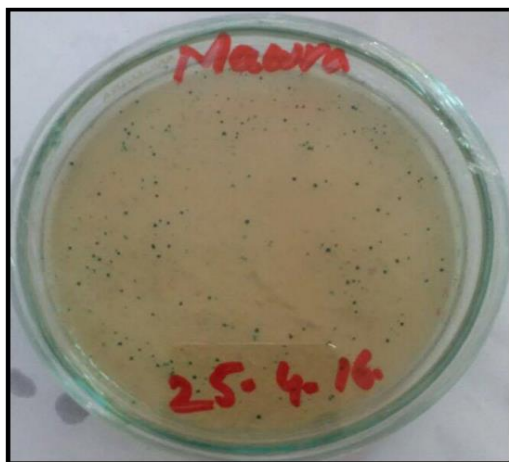


Fig. 6. Appearance of blue-white colonies after transformation of competent cells.

### Isolation of Plasmid DNA by Alkaline Lysis Method

After the incubation of liquid culture, plasmid DNA was isolated from cultured transformed *E.coli* cells by alkaline lysis method devised by Birnboim and Doly (1979) with certain modifications. Pellet of plasmid DNA was dissolved in 40 µl of R40. The quantity & quality of isolated plasmid DNA was assessed by 0.8 % agarose gel electrophoresis as shown in Figure 7.

### Isolation of plasmid DNA by FavorPrep™ Plasmid DNA Extraction Mini Kit

Plasmid DNA was also isolated from cultured transformed *E.coli* cells by FavorPrep™ Plasmid DNA Extraction Mini Kit (Thermo Scientific, USA). Pellet of plasmid DNA was dissolved in 40 µl of R40. The quality and quantity of isolated plasmid DNA was assessed by 0.8 % agarose gel.

### Confirmation of Recombinant Clones through Restriction Analysis

#### Restriction Analysis of Recombinant Plasmid DNA Isolated by Alkaline Lysis Method

Plasmid DNA was subjected to restriction digestion with endonucleases *EcoRI* and *HindIII* to cut plasmid at restriction sites present in pTZ57R/T cloning vector and the digested product was resolved on 0.8% agarose gel. It was observed that approximately 1500 bps was restricted out and back bone of approximately 2800 bps Fig 8.

#### Restriction Analysis of Recombinant Plasmid DNA Isolated by FavorPrep™ Plasmid DNA Extraction Mini Kit

Plasmid DNA was subjected to restriction digestion with endonucleases *EcoRI*, *HindIII*, *KpnI*, *MluI* and *XhoI* to cut plasmid at restriction sites present in pTZ57R/T cloning vector and the digested product was resolved on 0.8% agarose gel. It resulted in desired DNA fragments of approximately 4500 bps while restricted with *EcoRI* and *HindIII* alone while a desired fragment of approximately 1500 bp (insert) was restricted out when treated with *EcoRI* and *HindIII*, *KpnI* and *HindIII*, *MluI* and *XhoI*, respectively hence confirmed cloning of the desired gene in T/A cloning vector Fig 9.

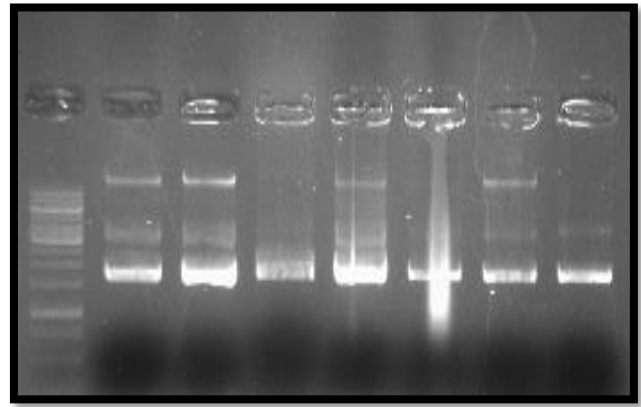
### Sequencing and *in Silico* Analysis of the Cloned Glucanase Gene

Sequencing analysis showed that T/A cloned glucanase gene was 1383 bps. Graphical representation of alignment was shown in figure 4.9.1. It shows alignment of *Glucanase* with the sequences of BLAST. Cloned sequence of *Glucanase* gene was amplified and its restriction map was found out using Restriction mapper (online software) at <http://www.restrictionmapper.org/>. Restriction mapper is an online tool that accepts DNA sequence and returns a textual map showing the positions of restriction endonuclease cut sites in the sequence. ExPASy translate tool (<http://web.expasy.org/translate/>) was used to find out the amino acid sequence. Largest open reading frame is the probable amino acid sequence of cloned DNA fragment. Phylogenetic tree shows the relationship among the selected database sequences and the query in a dendrograph. The distances for the tree view are derived from the pairwise local alignment between query and that of selected database sequences. Protein structure of cloned DNA fragment was predicted using **Phyre<sup>2</sup>** server (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) Fig 10.

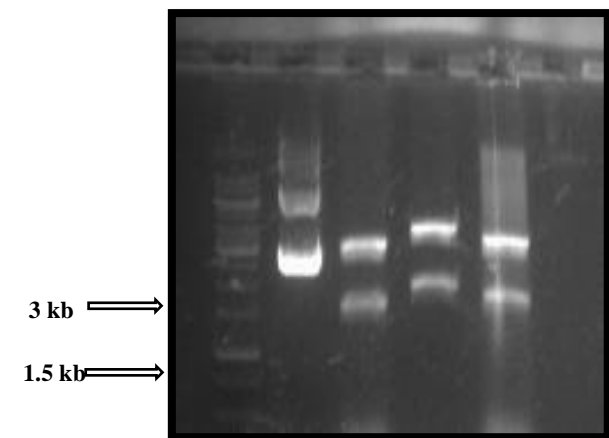
### Cloning of Glucanase Gene in Expression Vector pLH7000M

#### Isolation of Plasmid DNA by FavorPrep™ Plasmid DNA Extraction Mini Kit

Plasmid DNA was also isolated from cultured transformed *E. coli* cells by FavorPrep™ Plasmid DNA Extraction Mini Kit (Thermo Scientific, USA). Pellet of plasmid DNA was dissolved in 40 µl of R40. The quantity & quality of isolated plasmid DNA was assessed by 0.8 % agarose gel electrophoresis. Band was observed approximately 9000 bps Fig 11.



**Fig. 7.** Isolation of plasmid DNA by alkaline lysis method devised by Birnboim and Doly (1979) with certain modifications.



**Fig. 8.** Restriction analysis of *Glucanase* gene cloned in pTZ57R/T cloning vector. L stands for 1Kb DNA ladder and lanes 2, 3, 4, represent plasmid DNA restricted with *EcoRI* and *HindIII* and 1<sup>st</sup> lane represents undigested plasmid DNA.

#### Restriction of insert DNA (*Glucanase* Gene Cloned in T/A cloned vector)

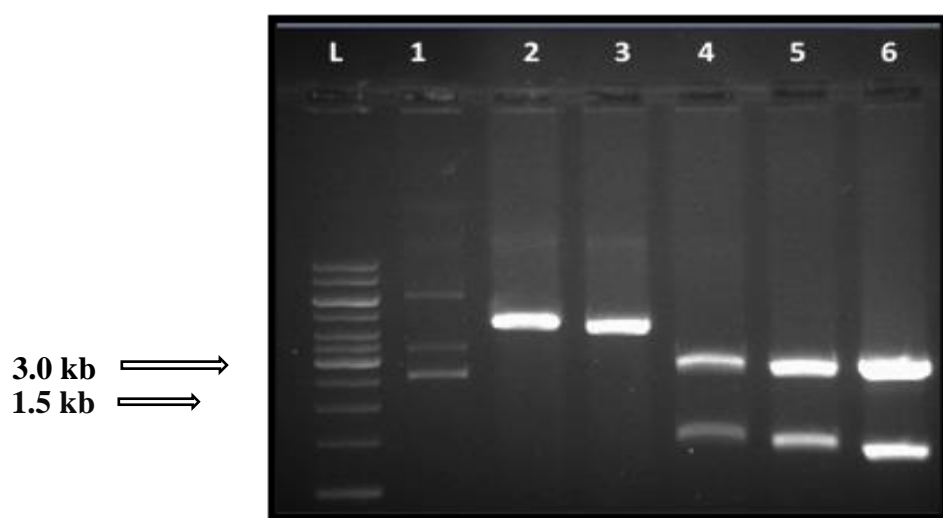
Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *XhoI* to cut plasmid at restriction sites present in pTZ57R/T cloning vector and pLH7000M vector the digested product was resolved on 0.8% agarose gel. It resulted in desired DNA fragments of approximately 1500 bps (insert) was restricted out and 2700 bps (vector backbone) Fig 12.

#### Restriction of pLH7000M Expression Vector

Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *XhoI* to cut plasmid at restriction sites present in pLH7000M expression vector and the digested product was resolved on 0.8% agarose gel. It resulted in two fragments of approximately 750 bps and 8000 bps Fig 13.

#### Ligation of pLH7000M Plasmid and Restricted *Glucanase* Gene

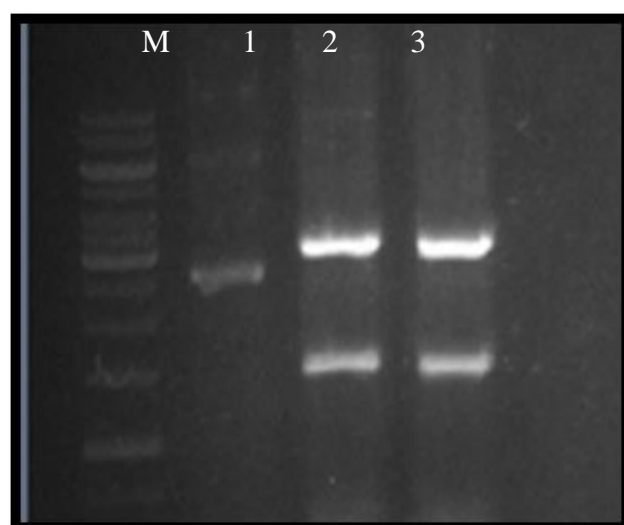
Restricted fragment of *Glucanase* from T/A clone and pLH7000M was ligated. The ligation mixture was incubated at 16°C for 16 hours followed by transformation in the *E. coli* bacterial cells or stored at -20°C Fig 14.



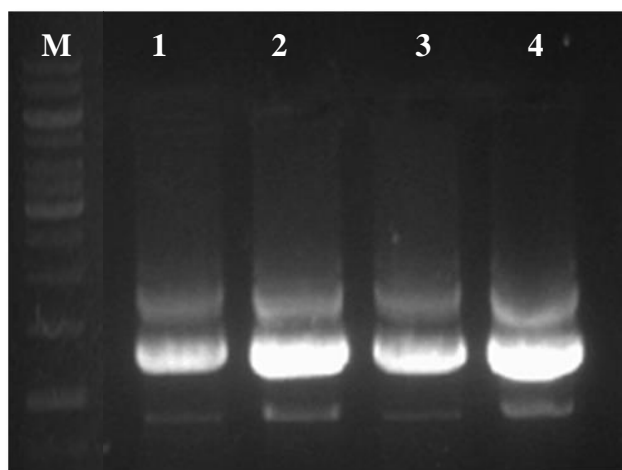
**Fig: 9:** Restriction analysis of recombinant T/A plasmid DNA having Glucanase gene as insert. L represents 1 Kb DNA ladder, lanes 2, 3 represent restricted plasmid DNA with restriction enzymes *EcoRI*, 3<sup>rd</sup> lane represents restriction with *HindIII*, lane 4 represents digestion with *EcoRI* and *HindIII*, lane 5 represents restriction with *HindIII* and *KpnI* while lane 6 represents reaction with *MluI* and *XhoI* respectively.



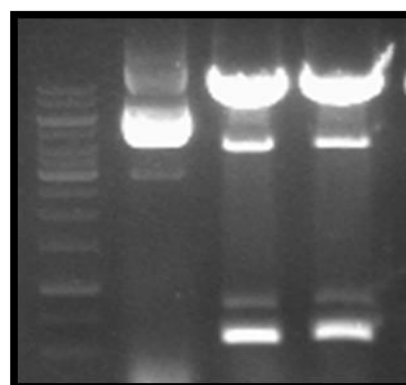
**Fig: 10.** Predicted structure of protein for Glucanase Image coloured by rainbow N  $\rightarrow$  C terminus Model dimensions (Å): X: 56.497 Y: 60.512 Z: 49.763.



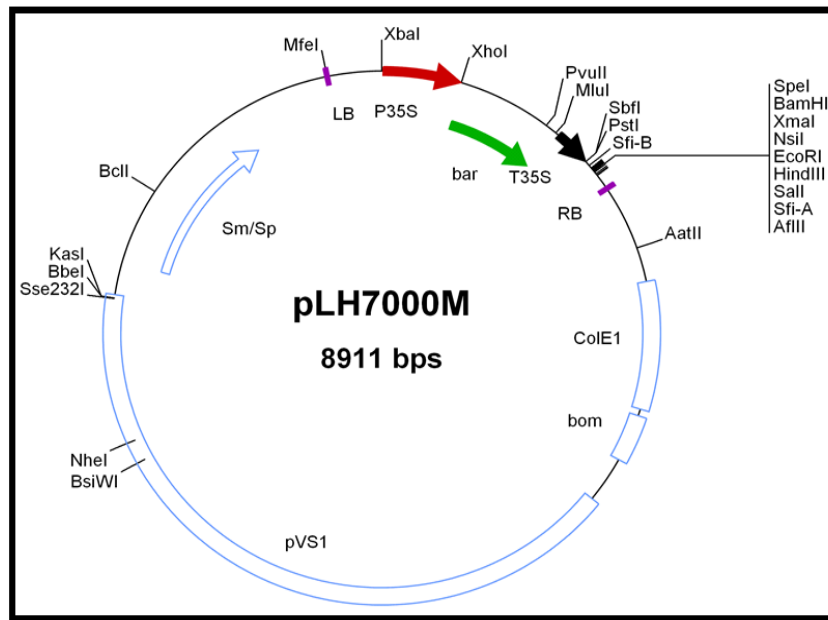
**Fig: 12.** Restriction analysis of T/A clone having Glucanase gene as insert. L represents 1 Kb DNA ladder, lanes 2, 3 represent restricted plasmid DNA with restriction enzymes *MluI* and *XhoI* and lane 1 represents undigested plasmid DNA.



**Fig: 11.** Isolation of plasmid DNA by FavorPrep™ Plasmid DNA Extraction Mini Kit.



**Fig: 13.** Restriction of pLH7000M vector with *MluI* and *XhoI*. The resultant fragment of around 8000 bps was used as vector for the cloning of regulatory sequences at the far ends of clone glucanase gene.



**Fig: 14.** Map of pLH7000M expression vector showing multiple cloning sites and selection markers.

#### Isolation of Plasmid DNA

After the incubation of liquid culture, plasmid DNA was isolated from cultured transformed *E.coli* cells by alkaline lysis method devised by Birnboim and Doly (1979) with certain modifications. Pellet of plasmid DNA was dissolved in 40  $\mu$ l of R40. The quality and quantity of isolated plasmid DNA was assessed by 0.8 % agarose gel electrophoresis. Band of approximately 1kb was observed.

#### Restriction Analysis of Recombinant Plasmid DNA Isolated by Alkaline Lysis Method

Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *HindIII* to cut plasmid at restriction sites present in pLH7000M expression vector and the digested product was resolved on 0.8% agarose gel. It was observed that approximately 750 base pairs was restricted out and back bone of approximately 8000 bps Fig 15.

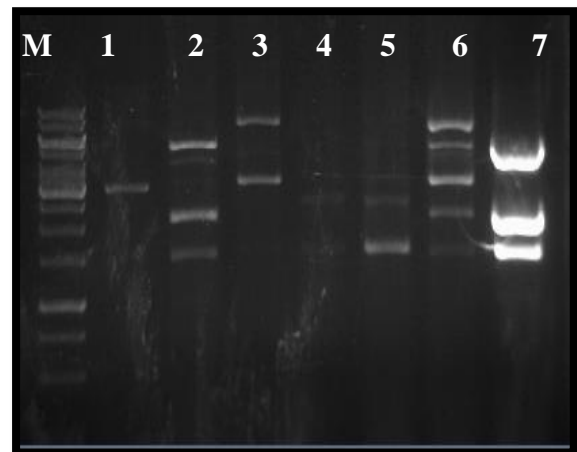
#### Restriction Analysis of Recombinant Plasmid DNA Isolated by FavorPrep™ Plasmid DNA Extraction Mini Kit

Plasmid DNA was subjected to restriction digestion with endonucleases *MluI* and *XhoI* to cut plasmid at restriction sites present in pLH7000M expression vector and the digested product was resolved on 0.8% agarose gel. It resulted in desired DNA fragments of 8000 bps (vector backbone) and approximately 1500 bp (insert), hence confirmed cloning of the desired gene in pLH7000M expression vector Fig 16.

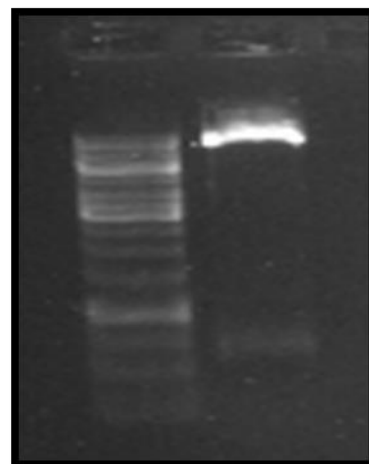
#### In silico Analysis of Glucanase Gene

##### Phylogenetic Tree

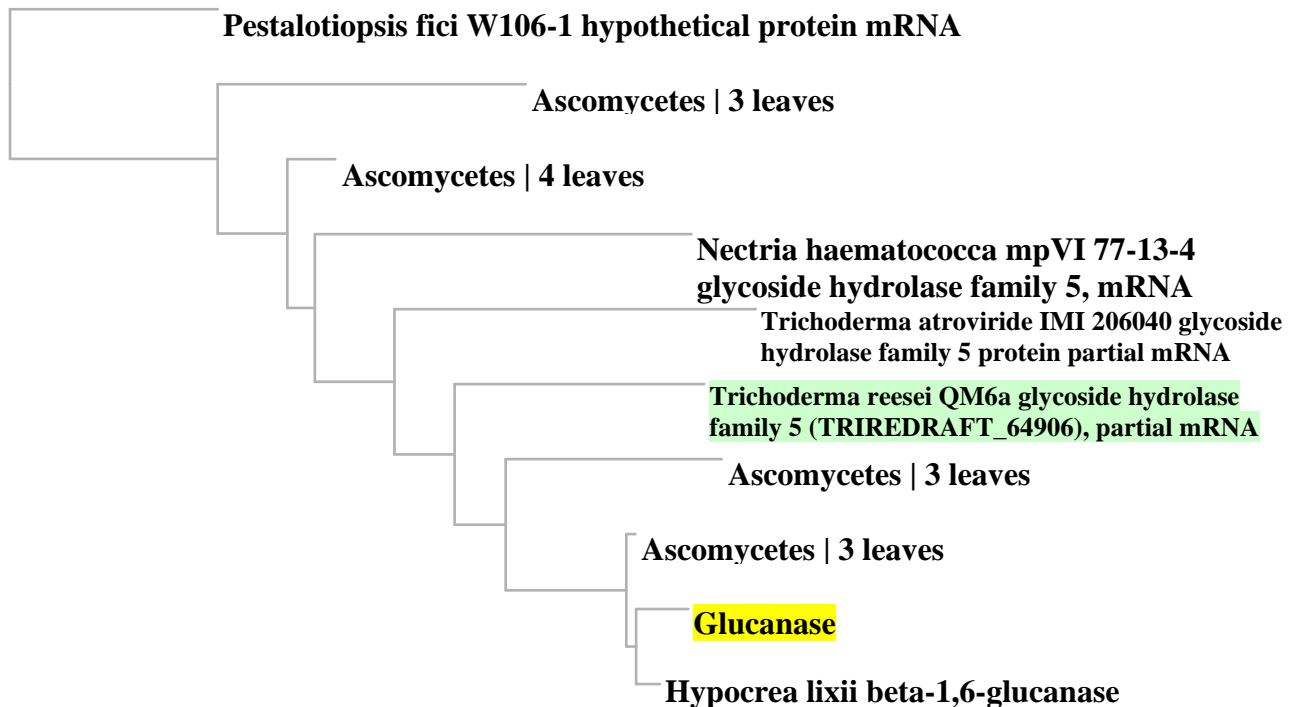
Phylogenetic tree shows the relationship among the selected database sequences and the query in a dendrograph. The distances for the tree view are derived from the pairwise local alignment between query and that of selected database sequences Fig 17.



**Fig: 15.** Restriction analysis of *Glucanase* gene cloned in pLH7000M expression vector. L for 1KB DNA ladder, 2, 3, 4, 5, 6, 7 represents plasmid DNA restricted with *HindIII* and *XhoI* and 1 represents undigested DNA.



**Fig: 16.** Restriction analysis of recombinant plasmid DNA having *Glucanase* gene as insert.



**Fig: 17.** Phylogenetic tree showing relatedness of the glucanase isolated for indigenous *Trichoderma* strains and the glucanase genes already reported.

## DISCUSSION

Increased productivity of crops is necessity of the day, to feed rapidly increasing population and to overcome the dilemma of decreasing agricultural land. Insect pests and diseases are the major obstacle in this regard as more plant diseases cause up to 12 percent losses in agricultural production per year (Fokunang *et al.*, 2004) aggravating the shortage of food where at least 800 million people are poorly feed. Fungicides are used in huge quantities in order to manage disastrous fungal diseases. These hazardous chemicals get accumulated in the soil and food and have perilous effects on human health and environment. Another aspect is that they may result in the development of resistant pathogen. To tackle these problem, efficient alternatives are being investigated & explored as use of biocontrol agent seems to be a hopeful approach. In addition to other biocontrol agents, *Trichoderma harzianum* has proved to be very effective in controlling plant pathogens (El-Ashmony *et al.*, 2022). Various mechanisms have been evolved in *Trichoderma* spp. such as antagonism for nutrients and space mycoparasitism, inactivation of the pathogens enzymes, production of inhibitory compounds and induced resistance. The mycoparasitism involves the degradation of host's cell wall with aid of extracellular enzymes for example glucanases and chitinases which be able to degrade the fungal cell wall, permitting parasitic hyphae to piece the host cells (Elad, 1995). The enzymes purified from *Trichoderma* are significantly superior in antifungal activity than glucanolytic and chitinolytic enzymes derived from other source (Dhaver *et al.*, 2022).

*Trichoderma harzianum* competes for space and nutrients with phytopathogens. Mycoparasitic ability of *Trichoderma harzianum* make them most successful

colonizer of substrate than pathogens. Similarly, they also produce inhibitory compounds that inactivate the enzymes produced by pathogens and induce resistance in plants. Thus, *Trichoderma harzianum* is an efficient biocontrol agent having parasitic and antagonistic activity because of its enzymatic and other biochemical weapons. These metabolites can be used as bio-fungicides to protect against pathogenic fungi (Yadav *et al.*, 2023).

Inspite of immense interest in biological control agents, the utilization of these agents is limited owing to their inefficient field performance (Graham and Sticklen, 1994). The introduction of disease resistant genes in plants is a promising alternative to be incorporated in plants for disease management. Though, traditional breeding has been contributed a lot but has certain limitations. Genes encoding for resistance against fungal disease (Glucanases, Chitinases, and Proteases etc.) are being incorporated into crop plants by genetic engineering. In developing disease resistant transgenic plants, a crucial step in the recognition of an efficient source and then introduction into the target crops (Rodrigues *et al.*, 2023).

In the current investigation a full-length endoglucanase gene from *Trichoderma harzianum* was isolated a 1383 bps long specific band was successfully amplified from *Trichoderma harzianum* chromosomal DNA, which was confirmed by sequencing. This glucanase gene was sub-cloned into pTZ57R/T cloning vector 1383 bps clones were confirmed via restriction analysis & PCR. Finally, the clone was examined by sequencing and analyzed by using various Bioinformatics tools. The DNA fragment was recognized as  $\beta$ -1, 6 endoglucanase, and 98 % sequence homology was found in sequence databases. The nucleotide sequence was found to have 98 percent homology with known sequences in database. Further, particular restriction sites were engineered at the far ends

of glucanase gene for the cloning of regulatory sequences. CAMV35S promoter and terminator were cloned at its 5'end and 3'end respectively. The developed plant transformation vector will be used for engineering plant genome with glucanase gene and will be initially characterized in the model tobacco plant.

### Conclusion

Owing to the direct fungicidal impact of glucans, the study was planned for the isolation, cloning and characterization of  $\beta$ -1, 6-glucanase gene from *Trichoderma harzianum* validated *Trichoderma* isolate were grown on PDB (Potato Dextrose Broth) medium. Genomic DNA was isolated by using modified CTAB method. Then *Trichoderma harzianum* was characterized at molecular level by using specific primers.  $\beta$ -1, 6-glucanase gene was isolated by using gene specific primers subjected to PCR. The amplified product was purified and ligation was done into pTZ57R/T cloning vector. Cloned fragment was sequenced and resultant sequence was analyzed by using various bioinformatics tools i.e., Blast, Restriction mapper. It was found to have more than 98% homology with glucanase sequences reported in literature. Particular restriction sites were engineered at the far ends of glucanase gene for the cloning of regulatory sequences. CAMV35S promoter and terminator were cloned at its 5'end and 3'end respectively. The developed plant transformation vector will be used for engineering plant genome with glucanase gene and will be initially characterized in the model tobacco plant.

### Acknowledgement

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## Supplementary Data

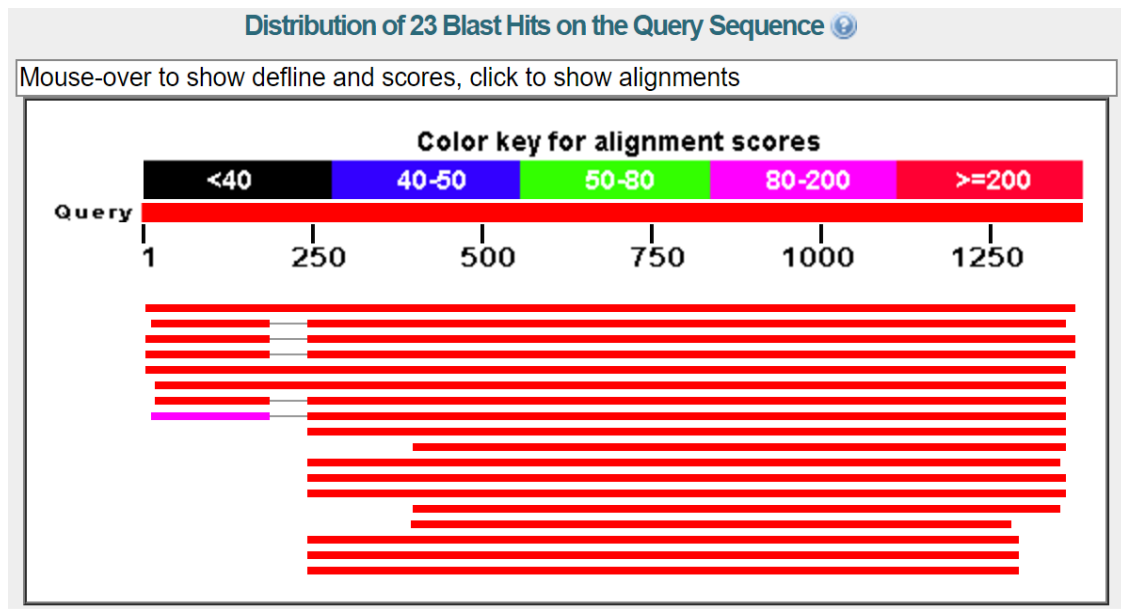
### Sequencing and *in silico* analysis of the cloned Glucanase gene

#### Nucleotide sequence of Glucanase

Sequencing analysis showed that T/A cloned glucanase gene was 1383 bps.

```
GCCTCGAGCCATCAAGATGAAGTACCCCATCGTTGCTCCGGCTATTCTCGCCGGCACC GCCTTTGCGTGGCTGCCTCAA
GACCGCGACCTGAAGGCCCTTCAACCAGACCGCTCGTTTTGAGCAGCTTGCCAAGCGCTTTGAGCCTGCGCTTGCTTCTG
GTATACCAAGATCCGTGGTGTCAACTTTGGTGGTAAGTGAATGTCTTATTTTCTGGAAGATTTAGAGAGTCAGGCTAAT
ATGATAAAGGATGGCTTATCAGCGAGCCATGGATGATGTCCAATGAGTGGAACAACAACATGGGCTGCAACGGTGCCG
CCTCTGAGTTTCGATTGTATGCGCAACAACACTACATGGGCAGCAAGCGTGCGACCGGAAACACCAAGTTCAGAACCACT
ACAGGGACTGGATTAACCCCGCCACCGTTTCAGTCTGTTACGATGTGCGCTTGAACACTATCCGTATCCCCATTGGCTAC
TGGTCCTACAATGCCATTGTGCGACACGGCTAGCGAGCCCTTTGCCGATGGCAACCTCCAGCTTCCGTATCTTGATGCTGT
TGTTTCAGAAGGCCGCTGATCTCGGAATCTACGTATCATTTGATCTTACGGTGCCCCCTGGCGGTCAGCAGCAGGACGCC
TTCACCGGCCAGAACCCCAACCCCGCGGGTTTCTACAACCTACATCGACTACGGCCGTGCTGAGAAGTGGCTGTCTTGGA
TGACCAACCGCATCCACACCAACCCTGCATACTCGACTGTTGGTATGATTGAGGTTCTCAACGAGCCCGTCTCCAGACA
CGACGGAGGTGGTTCGCTACCCTGCTCCTGGCCAGGACCCAAGCATGGTCCAGACCTACTACCCCGGCCCTCAAGGCT
GTGCGTGATGCCGAGGCTGCGCTGAACGTCCCAAGCAACAAGAAGCTGCACGTGCAGTTCATGTCCAGCAAGTGGGAT
TCTGGTGATCCTCGCAGCAACGCGCCGTCAAGAACGACCCCATGGTTGGCTTTGACGACCACAACACTACATTGGCTTTGC
CCTTAGCAACACTGGCGACCACTCTCTCATGACAGTGCATGCACTGACTCTCGTGTGCTGAACGGCCAGGACTTT
GCCATCACTGGCGAGTGGAGCATGACTTCTGGTGTGACTGGCATGGCGGAACTTCTTACGAAGTTCTTACAGCTC
AGCAGCAGCTGTATGAGTCCCCCGGAATGGACGGATGGATCTACTGGACCTGGAAGACCGAGCTGAACGACCCCGAT
GGACCTACTCTTATGCTACCTACCTCAACTACATCCCAACTAACGCCGCTGCCCTGCAGCAGAACGTTTACCAGGATGT
CTGCGCTGGATACAGGTAAATCACGAGTGATTGACGCGTGC
```

#### BLAST results of *Glucanase* gene



Graphical representation of alignment was shown in figure S1. It shows alignment of *Glucanase* with the sequences of BLAST (supplementary data).

Figure S1. Graphical representation

#### Amino acid sequence

ExPASy translate tool (<http://web.expasy.org/translate/>) was used to find out the amino acid sequence. Largest open reading frame is the probable amino acid sequence of cloned DNA fragment.

```
MKYPIVAPAILAGTAFWLPQDRDLKAFNQATARFEQLGKRFEPALASGITKIRGVNFGGK*MSYFLEDLESQANMIKDGLSAS
HG*CPMSGTTTTWAATVPPLSSIVCATTTWAASVRPETPSSRTTGTGLTPPFSLFTMSA*TLVSPLATGPTMPLSTRLASPLP
MATSSFRILMLLFRRLISESTSSLIFTVPLAVSSRTPSPARTPTPRVSTHTTTAVLRSGCLG*PTASTPTLHTRLLV*LRFSTSPS
PDTTEVVATLLARTQAWSRPTTPAPSRLCVMPLRL*TSQATRSTCTSSCPASGILVILAATRRQERPHGWL*RPQLHWLCP*Q
HWRPVLSHAQCMH*LSCRERPLCHHWRVEHDFWC*LAWRKLLEVLHSSAAV*VPRNGRMDLLDLEDRAERPPMDLLL
CYLPQLHPN*RRCPAAERLPGCLRWIQVNHE*LTRX
```



Sbjct	1688	TGCCCTTAGCAACACTGGCGACCAGTACTCTCTCATGCACAGCGCCTGCACTGACTCTCG	1747
Query	1085	TGTCGTGAACGGCCAGGACTTTGCCATCACTGGCGAGTGGAGCATGACTTCTGGTGTTGA	1144
Sbjct	1748	TGTCGTCAAGCGCCAGGACTTTGCTATCACTGGCGAGTGGAGCATGACCTCTGGTGCCGA	1807
Query	1145	CTGGCATGGCGGAAACTTCTTCACGAAGTTCTTCACAGCTCAGCAGCAGCTGTATGAGTC	1204
Sbjct	1808	CTGGCATGACGGAAACTTCTTCACCAAGTTCTTCACAGCTCAGCAGCAGCTGTATGAGTC	1867
Query	1205	CCCCGGAATGGACGGATGGATCTACTGGACCTGGAAGACCGAGCTGAACGACCCCCGATG	1264
Sbjct	1868	TCCTGGAATGGACGGATGGATCTACTGGACCTGGAAGACCGAGTTGAACGACCCCCGATG	1927
Query	1265	GACCTACTCTTATGCTACCTACCTCAACTACATCCCAACTAACGCCGCTGCCCT---GCA	1321
Sbjct	1928	GACCTACTCTTATGCTACCTACCTCAACTACATCCCAACCAACGCTGCTGCCCTGCAGCA	1987
Query	1322	GCAGAACGTTTACCAGGATGTCTGCGCTGGATACAGGTAAATCACGAGTGATTG	1375
Sbjct	1988	GCAGAACGTTTACCAGGATGTCTGCTCTGGATACAGGTAAATCACGAGTGATTG	2041

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Length=1290 Score= 1959 bits (988), Expect = 0.0 Identities = 1085/1116 (97%), Gaps = 1/1116 (0%)  
Strand=Plus/Plus

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Sbjct	175	GGATGGCCTATCAGCGAGCCATGGATGATGTCCAATGAGTGGAACAACGACATGGGCTGC	234
Query	307	AACGGTGCCGCCTCTGAGTTCGATTGTATGCGCAACAACCTACATGGGCAGCAAGCGTGCG	366
Sbjct	235	AACGGTGCTGCCTCTGAGTTCGATTGTCATGCGCAACAACCTACATGGGCAGCAAGCGTGCT	294
Query	367	ACCGGAAACACCAAGTTCAGAACCACTACAGGGACTGGATTAACCCCGCCACCGTTCAG	426
Sbjct	295	ACCGGAAACACCAAGTTCAGAACCACTACAGGGACTGGATTAACCCCGCCACCGTTCAG	354
Query	427	TCTGTTCCAGATGTGCGGCTTGAACACTATCCGTATCCCCATTGGCTACTGGTCCTACAAT	486
Sbjct	355	TCTGTTCCAGATGTGCGGCTTGAACACTATCCGTATCCCCATCGGCTACTGGTCCTACAAC	414
Query	487	GCCATTGTGCGACACGGCTAGCGAGCCCTTTGCCGATGGCAACCTCCAGCTTCCGTATCTT	546
Sbjct	415	GCCATTGTGCGACACGGCTAGCGAGCCCTTTGCCGATGGCAACCTCCAGCTTCCGTACCTT	474
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Sbjct	475	GATGCTGTTGTTTCAGAAGCCGCTGATCTCGGAATCTACGTCATCATTGATCTTCACGGT	534
Query	607	GCCCCGTGCGGTCAGCAGCAGGACGCCTTCACCGGCCAGAACCCCAACCCCGCGGGTTTC	666
Sbjct	535	GCCCCGTGGTGGTCAGCAGCAGGACGCCTTCACCGGCCAGAACCCCAACCCCGCGGGTTTC	594
Query	667	TACAACTCATACGACTACGGCCGTGCTGAGAAGTGGCTGTCTTGGATGACCAACCGCATC	726
Sbjct	595	TACAACTCGTACGACTATGGCCGTGCTGAGAAGTGGCTGTCTTGGATGACCAACCGCATC	654
Query	727	CACACCAACCTGTCATACTCGACTGTTGGTATGATTGAGGTTCTCAACGAGCCCGTCTCC	786
Sbjct	655	CACACCAACCTGCTTACTCGACTGTTGGTATGATTGAGGTTCTCAACGAGCCTGTCTCC	714
Query	787	AGACACGACGGAGGTGGTCGCTACCCTGCTCCTGGCCAGGACCCAAGCATGGTCCAGACC	846
Sbjct	715	AGACATGATGGAGGTGGTCGCTACCCTGCTCCTGGCCAGGACCCAAGCATGGTCCAGACC	774
Query	847	TACTACCCCGCGCCCTCAAGGCTGTGCGTGATGCCGAGGCTGCGCTGAACGTCCCAAGC	906
Sbjct	775	TACTACCCCGCGCCCTCAAGGCTGTGCGTGATGCCGAGGCTGCGCTGAACGTCCCAAGC	834
Query	907	AACAAGAAGCTGCACGTGCAGTTCATGTCCAGCAAGTGGGATTCTGGTGATCCTCGCAGC	966
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Query	967	AACG-CGCCGTCAAGAACGACCCCATGGTTGGCTTTGACGACCACAACCTACATTGGCTTT	1025
Sbjct	895	AACGCCGCCGTCAAGAACGACCCCATGGTTGGATTGACGACCACAACCTACATTGGCTTT	954
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Query	1086	GTCGTGAACGGCCAGGACTTTGCCATCACTGGCGAGTGGAGCATGACTTCTGGTGTGAC	1145
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Query	1146	TGGCATGGCGGAAACTTCTTCACGAAGTCTTCACAGCTCAGCAGCAGCTGTATGAGTCC	1205
Sbjct	1075	TGGCACGACGGAAACTTCTTCACCAAGTCTTCACAGCTCAGCAGCAGCTGTATGAGTCT	1134
Query	1206	CCCGGAATGACGGATGGATCTACTGGACCTGGAAGACCGAGCTGAACGACCCCGATGG	1265

```

Sbjct 1135 CCTGGAATGGACGGATGGATCTACTGGACCTGGAAGACCGAGCTGAACGACCCCTCGATGG 1194
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|||||
Sbjct 1195 ACCTACTCTTATGCTACCTACCTCAACTACATCCCAACTAACGCCGCTGCCCTGCAGCAG 1254
Query 1326 AACGTTTACCAGGATGTCTGCGCTGGATACAGGTAA 1361
|||||
Sbjct 1255 AACGTTTACCAGGATGTCTGCTCTGGATACAGGTAA 1290

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Score = 325 bits (164), Expect = 3e-84 Identities = 173/176 (98%),  
Gaps = 0/176 (0%) Strand=Plus/Plus

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|||||
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Sbjct 61 CAAGACCGCGACCTGAAGGCCTTCAACCAGACCGCTCGTTT GAGCAGCTTGGCAAGCGC 120
Query 137 TTTGAGCCTGCGCTTGCTTCTGGTATCACCAAGATCCGTGGTGTCAACTTTGGTGG 192
|||||
Sbjct 121 TTTGAGCCTGCACTTGCTTCTGGTATCACCAAGATCCGTGGTGTCAACTTCGGTGG 176

```

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Length=1330 Score = 1899 bits (958), Expect = 0.0 Identities = 1088/1130 (96%), Gaps = 1/1130 (0%)  
Strand=Plus/Plus

```

Query 247 GGATGGCTTATCAGCGAGCCATGGATGATGTCCAATGAGTGAACAACAACATGGGCTGC 306
|||||
Sbjct 191 GGATGGCTTATCAGTGAGCCATGGATGATGTCCAATGAGTGAACAACAACATGGGCTGC 250
Query 307 AACGGTGCCGCTCTGAGTTCGATTGTATGCGCAACAACATACATGGGCAGCAAGCGTGCG 366
||| |||
Sbjct 251 AACAAATGCTGCCTCTGAGTTCGATTGCATGCGCAACAACACTACTCGGGCAGCAAGCGTGCG 310
Query 367 ACCGGAACACCAAGTTCAGAAACCACTACAGGGACTGGATTAACCCCGCCACCGTTCAG 426
|||||
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|||||
Sbjct 371 TCTGTTACAGATGTGCGGCTGAACACTATCCGTATCCCCATCGGCTACTGGTCCTACAAC 430
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|||||
Sbjct 551 GCCCCCTGGTGGTCAGCAGCAGGACGCCCTTACCGGCCAGAACCCCAACCCCGCGGGTTTC 610
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|||||
Sbjct 611 TACAACCTGATGACTATGGTGTGCTGAGAAGTGGCTGTCTTGATGACCAACCGCATC 670
Query 727 CACACCAACCCCTGCATCTGACTGTTGGTATGATTGAGGTCTCAACGAGCCCGTCTCC 786
|||||
Sbjct 671 CACACCAACCCCTGCCTACTCGACTGTTGGTATGATTGAGGTCTCAACGAGCCCTGTCTCC 730
Query 787 AGACACGACGGAGGTGGTCGCTACCCTGCTCCTGGCCAGGACCAAGCATGGTCCAGACC 846
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```

```

Query 1146 TGGCATGGCGGAAACTTCTTCACGAAGTTCTTCACAGCTCAGCAGCAGCTGTATGAGTCC 1205
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
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          ||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 1151 CCTGGAATGGACGGATGGATCTACTGGACCTGGAAGACCGAGTTGAACGACCCTCGATGG 1210
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Sbjct 1211 ACCTACTCTTATGCTACCTACCTCAACTACATCCCAACTAACGCCGCTGCCCTGCAGCAG 1270
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Sbjct 1271 AACGTTTACCAGGATGTCTGCGCTGGATTACAGGTAAATCACGAGTGATTG 1320

```

### Restriction mapping of cloned Glucanasegene

**Name:** Glucanase

**Conformation:** linear

**Overhang:** five\_prime, three\_prime, blunt

**Minimum Site Length:** 5 bases

**Maximum Number of Cuts:** all

**Included:** all commercial, prototypes only

**Noncutters:** AarI, AatII, AbsI, AflII, AgeI, AjuI, AlfI, Aloi, ApaI, ApaLI, ApoI, ArsI, AscI, AsuII, AvrII, BaeI, BamHI, BarI, BbvCI, BclI, BfiI, BglII, BsaBI, BsaXI, BsePI, BseRI, BseYI, BsmI, Bsp1407I, BspHI, BspMI, BsrDI, BstEII, BstXI, BtgZI, BtrI, BtsI, ClaI, DraII, Eam1105I, Eco31I, EcoNI, EcoRI, EcoRV, FseI, FspAI, HindIII, HpaI, KpnI, MauBI, MfeI, MmeI, NdeI, NotI, NruI, OliI, PacI, PaeI, PfoI, PmeI, PpiI, PshAI, PsiI, PI-PspI, PstI, PvuI, RsrII, SacI, SanDI, SapI, PI-SceI, SexAI, SfiI, SgfI, SgrAI, SgrDI, SmaI, SnaBI, SpeI, SrfI, Sse8387I, SspI, SwaI, Tsp45I, TstI, Tth111I, VspI, XbaI

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
<a href="#">BaiI</a>	TGGCCA	6	blunt	1	821
<a href="#">BsaAI</a>	YACGTR	6	blunt	1	921
<a href="#">BsrBI</a>	CCGCTC	6	blunt	1	110
<a href="#">Eco47III</a>	AGCGCT	6	blunt	1	134
<a href="#">MslI</a>	CAYNNNNRTG	6	blunt	1	14
<a href="#">NaeI</a>	GCCGGC	6	blunt	1	52
<a href="#">PmaCI</a>	CACGTG	6	blunt	1	921
<a href="#">PvuII</a>	CAGCTG	6	blunt	1	1193
<a href="#">ScaI</a>	AGTACT	6	blunt	1	1050
<a href="#">StuI</a>	AGGCCT	6	blunt	1	95
<a href="#">AccI</a>	GTMKAC	6	five_prime	1	494
<a href="#">AclI</a>	AACGTT	6	five_prime	1	1327
<a href="#">AflIII</a>	ACRYGT	6	five_prime	1	1376
<a href="#">AvaI</a>	CYCGRG	6	five_prime	1	3
<a href="#">Bpu10I</a>	CCTNAGC	6	five_prime	1	1029
<a href="#">BsmAI</a>	GTCTC	5	five_prime	1	786
<a href="#">DraII</a>	RGGNCCY	6	five_prime	1	825
<a href="#">Esp3I</a>	CGTCTC	6	five_prime	1	786
<a href="#">HgaI</a>	GACGC	5	five_prime	1	637
<a href="#">MluI</a>	ACGCGT	6	five_prime	1	1376
<a href="#">NarI</a>	GGCGCC	6	five_prime	1	857
<a href="#">NheI</a>	GCTAGC	6	five_prime	1	502
<a href="#">PpuMI</a>	RGGWCCY	7	five_prime	1	825
<a href="#">PspXI</a>	VCTCGAGB	8	five_prime	1	3
<a href="#">SalI</a>	GTCGAC	6	five_prime	1	493
<a href="#">TatI</a>	WGTACW	6	five_prime	1	1048
<a href="#">XhoI</a>	CTCGAG	6	five_prime	1	3
<a href="#">BglI</a>	GCCNNNNNGGC	6	three_prime	1	865
<a href="#">BseSI</a>	GKGCMC	6	three_prime	1	609
<a href="#">DrdI</a>	GACNNNNNGTCT	6	three_prime	1	1083
<a href="#">EciI</a>	GGCGGA	6	three_prime	1	1168
<a href="#">Eco57I</a>	CTGAAG	6	three_prime	1	110
<a href="#">HaeIV</a>	GAYNNNNNRCT	6	three_prime	1	272
<a href="#">Hpy99I</a>	CGWCG	5	three_prime	1	796
<a href="#">NmeAIII</a>	GCCGAG	6	three_prime	1	906
<a href="#">NspI</a>	RCATGY	6	three_prime	1	1072
<a href="#">PstI</a>	CTGCAG	6	three_prime	1	1321

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
<a href="#">SacII</a>	CCGCGG	6	three_prime	1	659
<a href="#">SphI</a>	GCATGC	6	three_prime	1	1072
<a href="#">TsoI</a>	TARCCA	6	three_prime	1	458
<a href="#">XcmI</a>	CCANNNNNNNNTGG	6	three_prime	1	1014
<a href="#">XmnI</a>	GAANNNTTC	6	blunt	2	95, 1173
<a href="#">AcyI</a>	GRCGYC	6	five_prime	2	629, 857
<a href="#">Cfr10I</a>	RCCGGY	6	five_prime	2	50, 637
<a href="#">NcoI</a>	CCATGG	6	five_prime	2	265, 988
<a href="#">StyI</a>	CCWWGG	6	five_prime	2	265, 988
<a href="#">TfiI</a>	GAWTC	5	five_prime	2	578, 946
<a href="#">XhoII</a>	RGATCY	6	five_prime	2	168, 1222
<a href="#">AgsI</a>	TTSAA	5	three_prime	2	100, 447
<a href="#">AlwNI</a>	CAGNNNCTG	6	three_prime	2	401, 1193
<a href="#">BcgI</a>	CGANNNNNNTGC	6	three_prime	2	496, 530
<a href="#">BciVI</a>	GTATCC	6	three_prime	2	469, 1344
<a href="#">BdaI</a>	TGANNNNNNTCA	6	three_prime	2	79, 113
<a href="#">BplI</a>	GAGNNNNNCTC	6	three_prime	2	766, 798
<a href="#">BsgI</a>	GTGCAG	6	three_prime	2	901, 943
<a href="#">FalI</a>	AAGNNNNNCTT	6	three_prime	2	236, 268
<a href="#">GsuI</a>	CTGGAG	6	three_prime	2	514, 768
<a href="#">HaeII</a>	RGCGCY	6	three_prime	2	136, 860
<a href="#">PflMI</a>	CCANNNNNNTGG	6	three_prime	2	284, 941
<a href="#">TaqII</a>	GACCGA	6	three_prime	2	1231, 1257
<a href="#">TspDTI</a>	ATGAA	5	three_prime	2	32, 918
<a href="#">HindII</a>	GTYRAC	6	blunt	3	181, 495, 1142
<a href="#">FauI</a>	CCCGC	5	five_prime	3	420, 651, 663
<a href="#">PleI</a>	GAGTC	5	five_prime	3	234, 1071, 1208
<a href="#">SfaNI</a>	GCATC	5	five_prime	3	537, 731, 867
<a href="#">SmlI</a>	CTYRAG	6	five_prime	3	3, 75, 862
<a href="#">BseMII</a>	CTCAG	5	three_prime	3	311, 683, 1197
<a href="#">Eco57MI</a>	CTGRAG	6	three_prime	3	110, 514, 768
<a href="#">HphI</a>	GGTGA	5	three_prime	3	154, 627, 964
<a href="#">SduI</a>	GDGCHC	6	three_prime	3	512, 609, 779
<a href="#">TauI</a>	GCSGC	5	three_prime	3	316, 568, 1311
<a href="#">CfrI</a>	YGGCCR	6	five_prime	4	639, 684, 819, 1094
<a href="#">CspCI</a>	CAANNNNNGTGG	7	three_prime	4	154, 169, 189, 204
<a href="#">Hin4I</a>	GAYNNNNNVTC	6	three_prime	4	239, 271, 1035, 1067
<a href="#">TspRI</a>	CASTG	5	three_prime	4	1043, 1070, 1079, 1118
<a href="#">AvaII</a>	GGWCC	5	five_prime	5	476, 825, 837, 1231, 1264
<a href="#">EcoP15I</a>	CAGCAG	6	five_prime	5	649, 652, 1215, 1218, 1350
<a href="#">MboII</a>	GAAGA	5	three_prime	5	227, 589, 1154, 1166, 1250
<a href="#">TspGWI</a>	ACGGA	5	three_prime	5	161, 445, 527, 809, 1231
<a href="#">EcoRII</a>	CCWGG	5	five_prime	6	609, 816, 821, 1096, 1233, 1333
<a href="#">BccI</a>	CCATC	5	five_prime	7	17, 35, 242, 514, 1116, 1213, 1255
<a href="#">FokI</a>	GGATG	5	five_prime	7	260, 282, 709, 723, 1231, 1281, 1350
<a href="#">BsrI</a>	ACTGG	5	three_prime	7	406, 478, 1043, 1046, 1118, 1149, 1233
<a href="#">BbvI</a>	GCAGC	5	five_prime	12	57, 133, 289, 365, 633, 873, 902, 974, 1199, 1202, 1298, 1331
<a href="#">TseI</a>	GCWGC	5	five_prime	12	70, 121, 302, 353, 621, 886, 915, 962, 1187, 1190, 1311, 1319



## Metabolomics, a Potential Way to Improve Abiotic Stresses Tolerance in Cereal Crops

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### ABSTRACT

Rapid Climate change is causing the highly diversified effect on both quantity and quality in extensive area crops. All abiotic stresses are based on ecological conditions due to any small change that can create a future challenge for us. Metabolomics is a very valuable approach and powerful tool that helps to understand plant reaction stimuli. A range of variable analytical approaches (GC-MS, LC-MS, CE-MS, NMR) has been utilized to inspect the action of the metabolites. It promotes recognizing and understand the markers of bio-stimulants action. Plants have developed complex systems to recognize external signals to adjust according to fluctuating environmental factors for their survival in stressful environments. Plants respond to abiotic stresses by producing various metabolites and by expression of certain stress related genes. Comprehensive data recognition and identification of complicated metabolites is a challenge however appropriate software tools application promotes the accuracy with big data-driven precision. The review articulates hurriedly the basic aspects relating to identifying the pathways through valuable tools in metabolomics.

**Key words:** Biotic Stress, Abiotic Stress, Metabolomic Assisted Breeding, Metabolic Pathways, Nuclear Magnetic Resonance Spectroscopy, Metabolites, Reactive Oxygen Species.

### INTRODUCTION

Metabolomics is a new and exciting approach in omics technologies that has recently gained traction. Crop enhancement techniques have been widely used. Abiotic stress research requires the use of metabolomics. Tolerance, pathogen resistance, robust ecotypes, and metabolomic assisted crop breeding are all examples of metabolomic assisted breeding. Until now, the development of modern metabolomics tools for crop improvement has made tremendous progress (Shulaev et al., 2008; Razzaq et al., 2019).

The identification and quantification of all low molecular weight metabolites in each organism, at a given developmental stage, and in each organ, tissue, or cell type has been identified as metabolomics (Fiehn, 2001; Arbona et al., 2014). The fields of metabolomics, genomics, transcriptomics, epigenomics, proteomics and phenomics have all seen significant advancements in recent years. These 'Omics' methods have improved the precision and efficiency of ongoing breeding programs in developing climate-smart and nutrition-rich germplasm, which is necessary to assure food security (Parry and Hawkesford, 2012). Metabolomics has emerged as a valuable technology for systematic profiling and comparison of metabolites in biological systems, with a

wide range of applications documented in plant sciences (De Vos et al., 2007). The plant kingdom comprises approximately 200,000 different metabolites, the vast majority of which are still unknown. Around 10000 secondary metabolites have been discovered in various plant species, according to estimates. The newly identified metabolites vary structurally. In plant biology, their biochemical properties and functions are extremely significant (Foiato and Stewart, 2018). Modern metabolomics platforms are being used to visualize complex biological pathways and uncover secret regulatory networks that regulate crop production and growth. Within biological systems, a metabolome is a complete collection of low molecular weight metabolites. The primary goal of metabolomics research is to identify and quantify small molecules (less than 1500 Da), as well as their chemical structure and association within an organism (Deborde et al., 2017). Plants metabolize a wide range of primary and secondary metabolites with a variety of chemical structures. Plants need primary metabolites for growth and development, but secondary metabolites serve a different function, and both types of metabolites are involved in plant stress response mechanisms (Hong et al., 2016). Understanding chemical signals as plants grow and evolve requires metabolomics (Sharma et al., 2018).

One of the modern world's key concerns is to deal with the sustainable production of food to feed an ever-increasing population. According to the Food and Agriculture Organization (FAO), by 2050, crop productivity must have doubled. The productivity forecast of existing crops, however, is not encouraging due to climate and environmental stress conditions (Zafar *et al.*, 2021; Zafar *et al.*, 2022). Several crops' productivity is estimated to collapse by roughly 50% by 2080 in many parts of the world (Zhang *et al.*, 2019). Climate change threatens global agriculture because changes in rainfall, heat waves, and global CO<sub>2</sub> levels cause a variety of abiotic stresses that have a negative effect on food production (Raza *et al.*, 2020). Cereal crops such as rice, maize, wheat, barley, sorghum, oat, and millet account for most of the world's food supply (Balmer *et al.*, 2013). Since both biotic and abiotic stress factors pose a threat to crop production sustainability, abiotic stresses have a greater influence on agricultural productivity of crops such as wheat, soybean, and maize than biotic ones. Indeed, typical climate-related elements like water, temperature, and soil salinity reduce crop output significantly over the world (Ciura and Kurk, 2018). Changes in various cellular metabolic processes, including as glucose, amino acid, and peptide metabolism, are directly linked to abiotic stressors. However, if the metabolic balance is upset, growth, development, and, as a result, yield may be harmed (Das *et al.*, 2017).

As a response, the development of sustainable agriculture techniques, along with more productive and stress-tolerant crop varieties carrying genetic features linked with environmental change adaptation (or high tolerance to abiotic stresses), will be required to sustainably grow high-yielding crops under more demanding environmental conditions, either alone or in combination. (Furlan *et al.*, 2017; Zafar *et al.*, 2020). Intercropping can improve resilience to climate change through increased plant resource utilization i.e. space, nutrition, water, weeds, disease, and insect pest reduction (Manan *et al.*, 2022).

### Importance of Metabolomics in Plant Biology

Metabolomics is one of the emerging and fascinating approaches of omics tools that is now widely used for crop improvement. Metabolism is important for the study of abiotic stress tolerance, resistance to pathogens, strong ecotypes, and preparation of metabolic assisted breeding of various types of crops (Shulaev *et al.*, 2008).

To date, there have been significant advances in the development of modern metabolomic tools to study the metabolomic pathways of crops. There are 200,000 compounds, most of which are still unknown. An estimated 10,000 secondary metabolites have been discovered in various plant species (Foito and Stewart, 2018). The discovered metabolites are structurally different in biochemical properties and function and are considered very important in plant biology. Metabolome is a combination of with low molecular weight metabolites in biological systems. Metabolic research is primarily concerned with the identification and reduction of molecules as tiny, structural chemical molecules and their functions within the organism (Deborde *et al.*, 2017).

Many metabolites are unique and play an important role in controlling crop performance and nutritional quality. Plant growth in different environmental conditions

is affected by the synthesis of many metabolites. Ecological metabolism involves the study of the interaction of plants with their environment. This is done based on a careful estimate of the levels of metabolites under a specific plant environment to determine their effects on plant adaptation and any changes in their genetic makeup. It provides a platform for examining interactions between the environment and biology to measure plant performance in detail. Another important metabolic application, called ecological metabolism, is determined by analyzing the biochemical interactions of plants in transient and endemic systems. This biochemical nature explains many important environmental phenomena, such as the effects of parasitic loads, the disease, and its infection. It also helps to assess the various effects of non-living factors on the interaction between two traffic levels or the relationship between specific ones. Changes in the concentration of multiple metabolites may have mechanical implications for biochemical networks that explain the phenotypic and physiological feedback of plants to environmental fluctuations. The full potential of ecological transformation has not yet been explored. Biological and non-biological pressures adversely affect crop production and lead to a large reduction in global annual crop production. Metabolic tools can be integrated with mode tools such as genomics, transcriptomics, and proteomics to combat inanimate biotic stress in plants (Liu *et al.*, 2019).

Metabolomics help in analyzing the various metabolites of exotic and endogenous plants under extreme climatic pressure and is a key in understanding plant biology. The application of Omic-based strategies help us to understand the process of pressure regulation in plants. Plant metabolism consists of two types of metabolites: primary and secondary metabolites. The metabolic profile of primary and secondary metabolites provides extensive knowledge of the biochemical processes found during plant metabolism. Some primary and secondary metabolisms of plants can interact with very complex metabolic pathways. Successful identification, diagnosis, and diagnosis of these metabolites is possible through advanced metabolic instruments such as gas chromatography mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and nondestructive nuclear magnetism. Resonance spectroscopy (NMR) Primary metabolites are important for the biosynthesis of lipids, sugars, and amino acids in plants. They mediate the carboxylic acid cycle and the glycolysis cycle during photosynthesis, thus affecting plant growth and development. Changes in the synthesis of primary metabolites can lead to inactivation of photosynthetic function and failure to regulate asthmatic balance in plants. Primary metabolites leads to the production of secondary metabolites such as flavonoids, atropine, carotenoids and phytic acid. These are not essential for plant survival and are produced in response to various stress conditions such as high temperatures, cold, drought, salinity, and insect breeding. In addition, secondary metabolites include reactive oxygen species (ROS), antioxidants, and common enzymes (Seigler, 1998).

The plant metabolite consists of certain secondary metabolites such as phenolics (10,000 10,000), alkaloids (21,000), and terpenoids (> 25,000) that provide biotic / abiotic stress tolerance. Recently, many of these

compounds have been discovered as unique biomarkers that measure plant performance in stressful environments and serve as key components in many crop improvements programs. During plant ontogenesis, primary and secondary metabolites are formed by permanently complex biochemical reactions, so it is important to discover the unique metabolic biochemical processes present in plant organisms. Due to the diverse chemical synthesis, extensive production in cell components, and the complex nature of metabolites, no metabolic tool can be used for complete metabolism profiling. Although a number of methods of metabolome extraction and successful combination of analytical tools for successful metabolic profiling are widely used modern analytical operating systems for decoding the entire metabolism of any plant are still in the early stages (Rodziewicz *et al.*, 2014).

Metabolism has the potential to accelerate the selection of augmentative ingredients and a variety of elite products. Metabolic integration with modern plant genomic tools, such as genotype sequencing (GBS), genome genetic variables and whole genome sequencing, opens interesting horizons for crop improvement. Different cells, which are involved in different cellular processes, are used, thus revealing the whole anatomical structure of this cell.

### Abiotic Stresses and Responses of Metabolites in Energy Synthesis Pathway

Abiotic stresses negatively influence the homeostasis & photosynthesis metabolic pathways (Soares *et al.*, 2018; Yadav *et al.*, 2018). Photosynthesis is an energy-giving pathway in plants that directly affects the yield. Abiotic stress badly affects glucose synthesis as a basic source of cell functionality. These stresses cause to close the stomata through CO<sub>2</sub> reduction inside the cell. Also slows down the activity of Ribulose 1-5 bisphosphate carboxylase (RUBISCO) (Kohli *et al.*, 2017). Heavy metals (Cd, Hg, Cu, Pb, Ni, Zn) that are releasing from volcanoes cause soil pollution (Kumar *et al.*, 2020). Heavy metals are capable to change plant's essential elements to biomolecules that cause to reduce the energy supply level (Rascio and Navari-Izoo, 2011; Guo *et al.*, 2016). The shape of chloroplast is highly affected by Cd which reduces the photosynthesis rate (Najeeb *et al.*, 2011). Excess amount of Ni stops the formation of photosynthetic pigments and disrupts its apparatus (Soares *et al.*, 2019). Plants produce SOD, CAT, APX enzymes that promote antioxidant activity (Hasanuzzaman *et al.*, 2020). ROS accumulation can promote the activity of a resistant heavy metal gene by enhancing GHS, PCs, MTs, NA. Global warming promotes the temperature fluctuations worldwide that affect metabolic pathways like photosynthetic, growth pathways (Sharma *et al.*, 2020). There is highly disrupting stress to the photosynthetic system is heat stress (Mathur *et al.*, 2020). Chilling temperature also disturbs the energy synthesis pathway (Hou *et al.*, 2016). Low temperature damages the working of guard cells due to which stomata are closed and internal cell CO<sub>2</sub> concentration adversely affected (Allen and Ort 2001). In biochemical responses, the plant accumulates the MDA, H<sub>2</sub>O<sub>2</sub>. All of the enzymes and their activity are shown in fig1. Metabolomic studies conformed that transgenic rice ability to uptake more CO<sub>2</sub> and the high rate of photosynthesis process (Sakamoto and Matsuka, 2008). There is another abiotic stress that is

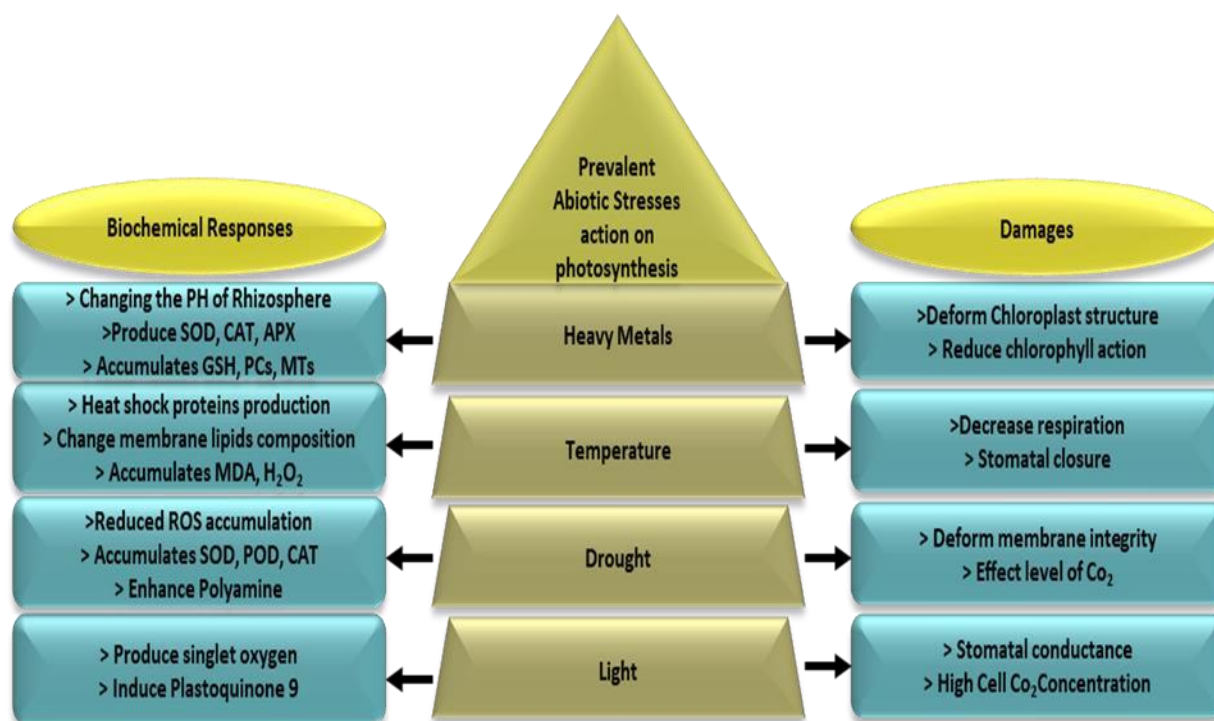
enhancing through increasing temperature is drought stress. Water is an essential ingredient in the photosynthesis mechanism, but drought causes water and CO<sub>2</sub> loss (Rivas *et al.*, 2016; Chen *et al.*, 2017). Light is considered a key stress factor that harms plant development (Wang *et al.*, 2017). Low light harms the rate of photosynthesis by activating the stomatal conductance and CO<sub>2</sub> concentration within the cell. In response to light plant induce plastoquinone-9.

Plants respond to environmental stresses by the multifaceted signaling pathway of molecules. Every systematic pathway in plants is based on metabolites activity due to which is essential to study for the maintenance and enhancement of quality and quantity. Plants produce several types of antioxidants by the activity of abiotic stresses like ascorbic acid, saponins, flavonoids, glycosylates, phenylpropanoid, and phenol amides (Nakabayashi and Saito, 2015). Flavonoids access and oxidative stress have Spatio - temporal correlation (Hernández *et al.*, 2009). It has been suggested that there is a correlation between metabolite accumulation and gene expression (Tohge and Fernie, 2020). Most of the sugar and water dissolving metabolites are stored in vacuoles act as antioxidants against abiotic stresses (Obata *et al.*, 2012). ROS (Reactive oxygen species) act as a biological marker but during any type of abiotic stress it accumulates. ROS induces hydrogen peroxide, superoxide anion radical, singlet oxygen, hydroxyl radical that cause protein, lipids damage. But sometimes enhances the stress signaling pathway to reduces the damage (Hasanuzzaman *et al.*, 2020). Their activity is based on the enzymatic and non-enzymatic antioxidant system so that play an important Osmo-protectant role during abiotic stresses.

### Assessment and Data Analysis Approaches in Metabolomics

In the plant kingdom, about 200,000 types of metabolites exist but still, a huge amount is unrevealed (Foito and Stewart, 2014). There are several biotic and abiotic stresses that directly or indirectly affect the plant metabolites. Metabolomics approaches promote the identification of these affected metabolites with other “omics” tools integration (Piasecka *et al.*, 2019). The observation, recognition, assessment, and data generation of these metabolites can achieve through various metabolomics tools (Che-Othman *et al.*, 2019).

There are numerous metabolites characterization and quantification tools but some most common are GC-MS, LC-MS, CE- MS and NMR. GC-MS a multipurpose analytical platform due to its high efficiency and reproducibility (Mastrangelo *et al.*, 2015). It can only be applicable for low molecular weight & volatile compounds. GC-MS approach analyzed Amino acids, Sugars (mono, di, trisaccharide's) and fatty acids, nucleotides, Ester (Koek *et al.*, 2006). Its implementations in target analysis metabolites and metabolomics (Nassar *et al.*, 2017). LC-MS are more applicable than GC-MS in the Clinical field for separation and analysis. The sample having a higher value than the threshold level re-analyzed by more particular LC-MS. LC-MS essay having the ability to multiplex numerous analytes in a single run with less cost (Pitt, 2009). CE- MS approach analyzed Amino acids, fatty acids, steroids, polar metabolites, and organic acids.



**Fig. 1:** Bio stimulants for Plant Growth and Mitigation of Abiotic Stresses: A Metabolomics Perspective.

SOD (Superoxide dismutase, an enzyme destroys the destructive molecule of oxygen within the cell).

CAT (Catalase, promote signaling within the cell and normalize cell activity by decomposition of hydrogen-peroxide).

POD (Peroxidase, A haem-containing enzyme that Destroy H<sub>2</sub>O<sub>2</sub>).

APX (Ascorbate peroxidase, Catalyze L-ascorbate & Hydrogen peroxide).

GSH (Glutathione, support in Fight that against free radicals).

MTs (Mesial temporal sclerosis, Stabilize homeostasis activity within the cell against environmental stresses).

MDA (Malondialdehyde, A aldehyde act as a biomarker to estimate the level of oxidative stress).

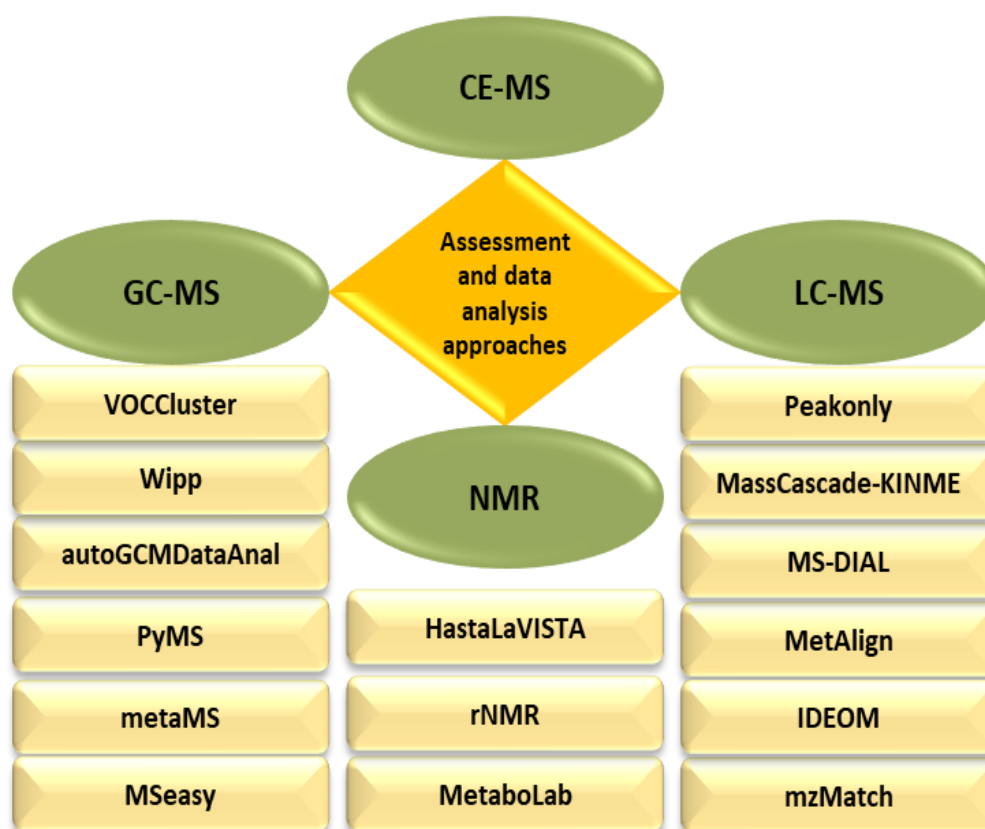
H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide, most stable predominantly producing reactive oxygen species ROs).

CE-MS a powerful tool that is used to allocate the charged compounds of a wide analytical range in biological samples (Indenburg *et al.*, 2014). In CE-MS poor concentration sensitivity was removed by porous tip sheathes interface and microbial interface because of metabolic coverage (Lindenburg *et al.*, 2014; Ramautar *et al.*, 2012). Its potential is strongly based on the reliability and sensibility of interfacing procedures like the approach of sheath liquid interface. A valuable step in CE-MS is interfaced versatility as a concentration of BGE changes with the type of interface (Chen *et al.*, 2017). Its implementations in target analysis and profiling of metabolites (Hou *et al.*, 2016). The UPLC-MS gives reservation time, accurate molecular mass, and MS-MS data of fragmentation for structural clarification of biomarkers and other metabolites (Jandrić *et al.*, 2013). NMR is high-throughput, non-destructive, inherently quantitative, automatable, large-scale studies of metabolomics than GC-MS and LCMS. It can be analyzed polar and non-polar metabolites more efficiently than other techniques. NMR supports imaging and analysis in observing metabolites samples by MRS and MRI. At last, NMR is one of the leading technologies than others in metabolomics (Jeong *et al.*, 2017).

#### Softwares or Tools for Data Analysis

Powerful software approaches are required for a large amount and variety of data generation in metabolomics (Cambiaghi *et al.*, 2016). A lot of tools such as GC-MS, LC-MS, NMR are used in metabolomic studies.

VOCcluster (use for organic compounds identification and density-based non-linear clustering), Wipp (data processing used to distinguish among peaks of real peaks and false peaks on the bases of algorithmic parameters) autoGCMDDataAnal (A software of data processing and data analysis), PyMS (its apply for parallel filtering among by row & by column data analyzing based on MPI) (O'Callaghan *et al.*, 2012) metaMS (its perform pseudospectral Analysis and keep away from alignment stage (Spicer *et al.*, 2017). MSeasy (A metabolite detector performing baseline rectification, smoothing, peak observation and deconvolution) tools are used for data analysis in GC-MS. Peakonly (it is an artificial neural network utilizer to recognize the region of interest) (O'Shea and Misra, 2020), MassCascade-KINME (it's an open-source data processing library) (LC-MS 3), MS-DIAL (an algorithmic base independent data processing), MetAlign (involve in preprocessing activities), IDEOM (an excel template used for identification and statical analysis of metabolites), mzMatch (Processing data based on PeakML design). GlyReSoft (use for glycans data processing) tools are used in LC-MS for data analysis (Maxwell *et al.*, 2012). The currently used approaches in NMR Data analysis are HastaLaVISTA (An R package that supports NMR Data analysis through Interactive Graphical port) rNMR (A preprocessing region-based interest approach) and MetaboLab (A batch processing that helps to detect the data more accurately through statistical analysis) (Ludwig and Günther, 2011). All tools are shown in Fig. 2.



**Fig. 2:** Basic tools for metabolomics data analysis.

### Workflow of Metabolomics

Now a days, abiotic stresses are the main cause of reduced agriculture yield potential in crop plants. Abiotic stresses can be defined as any change in plant growth conditions that affect normal plant growth, development, and physiology. Salinity, chilling, high temperature, drought, waterlogging, and heavy metals are the major abiotic stresses that affect plant growth (Raza *et al.*, 2019). All types of stresses result in significant reduction in yields quantity and quality (Hein *et al.*, 2016). Plants have a very active mechanism for sensing of stress signals and then cellular response are produced to overcome the stress. Both biotic and abiotic stresses produce variations in plant physiological and biochemical processes. When plants undergo abiotic stress, several phytochemicals are produced by the plants to resist against the stress. Different OMICS techniques has been used by scientists to improve the abiotic stresses tolerance in crop plants. Metabolomics is one of these OMIC techniques which is playing its role in improvement of plants. Different plant metabolites variations are under investigation to find out metabolites that can restore the normal plant homeostasis and other metabolic variations which are cause by abiotic stress (Khan *et al.*, 2019). Flow chart showing general steps which are involved in diagnosis and metabolomic assisted breeding for a biotic stress improvement in plants is shown in fig 3.

### Metabolomic Assisted Breeding

#### Drought Stress:

Drought is one of the most important abiotic stresses which adversely affect yield of crops worldwide. Plants

adopt different mechanisms such as abscission of leaves, increased nutrient uptake and reduction in plant leaf area. Similarly closure of stomata helps to reduce the transpiration activity which protect plants from water loss. All these physiological changes help plants to use the water more efficiently which affect the photosynthesis activity as there is reduced CO<sub>2</sub> and stomata closed (Chaves and Oliveira, 2004). Plants produce different polyamines like spermine, spermidine, and putrescine during drought stress (Bitrián *et al.*, 2015). Metabolomic profiling of 6 drought tolerant wheat lines shows that several important metabolites such as -aminobutyric acid (GABA), myo-inositol, threonine, proline, oxalic acid, malic acid, glucose, fructose, and sucrose are produced during drought stress (Marček *et al.*, 2019). High levels of amino acids like lysine, arginine and methionine are also observed when comparative metabolic analysis was done between drought susceptible and drought tolerant wheat lines (Michaletti *et al.*, 2018). These biomarkers can be used to screen drought tolerant lines. Key metabolites produced during different types of stress conditions by different crops are given in table 1.

LC-MS- and GC-MS-based metabolic profiling confirmed the differential accumulation of metabolites in young and matured leaves GC-MS based metabolic fingerprinting of 10 maize hybrids under drought stress revealed low concentration of maltose and erythritol while in rabinose and xylitol no change was observed. Drought tolerant maize lines have high levels of GABA, leucine, glycine, serine, alanine, and tryptophan (Obata and Fernie, 2012).

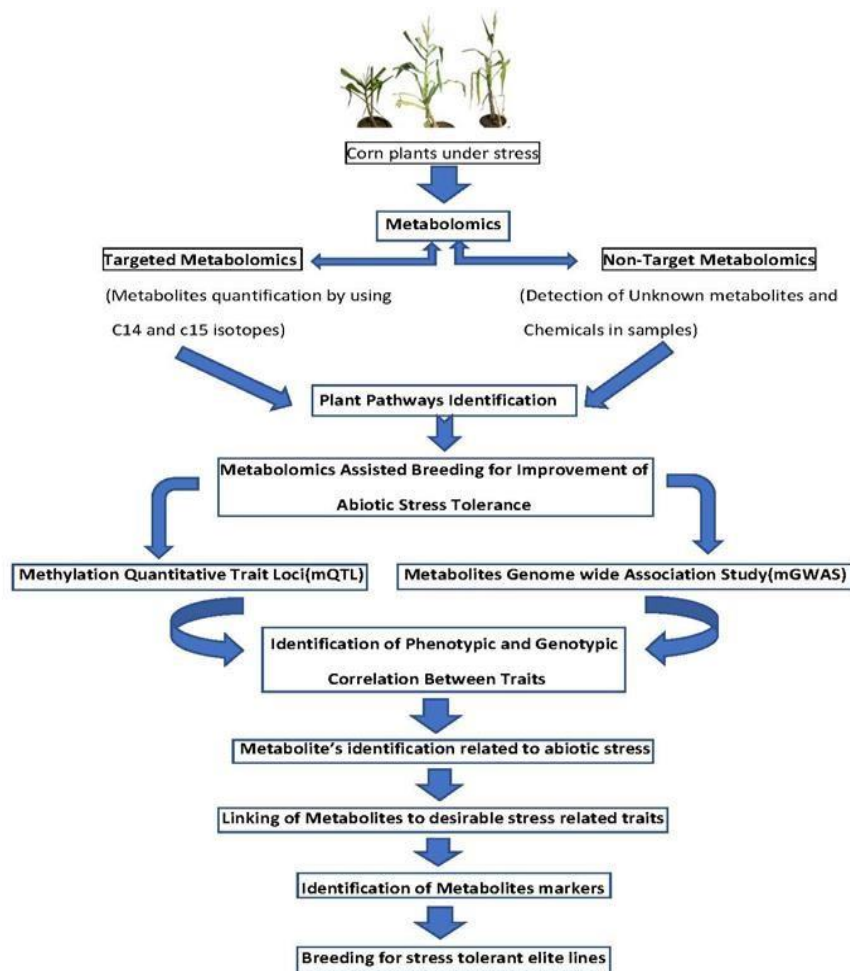


Fig. 3: Work flow diagram of Metabolomics.

Table 1: Major metabolites produced during different types of stress conditions by different crops

Crop name	Stress type	Specific tissue	Major Metabolites produced	Reference
<i>Zea mays</i>	Drought	Immature seeds	Metabolism of lipids, carbohydrates and glutathione cycle	Yang et al., 2018
<i>Triticum aestivum</i>	Drought	Leaf	Glutamine, serine, methionine, lysine and asparagine	Yadav et al., 2019
<i>Glycine max</i>	Drought	Leaf	Glutamine, GABA, allantoin, pinitol and myoinositol	Silvente et al., 2012
<i>Oryza sativa</i>	Salinity	Leaf	Mannitol and sucrose	Chang et al., 2019
<i>Sorghum bicolor</i>	Drought	Leaf	Sugars and sugar alcohols	Ogbaga et al., 2016
<i>Helianthus annuus</i>	Heavy metals	Roots	Fatty acids	Ibarra et al., 2019

### Salinity Stress Tolerance

High level of salinity affects nutrient uptake which results in poor growth and development of plants. High concentration of salts cause ion toxicity in plants which makes nutrients and water uptake more difficult for plants (Wu *et al.*, 2013). Different primary and secondary metabolites are produced by the plants under saline conditions. GC-MS base barley roots metabolic profiling indicated high levels of organic acid, proline, sucrose, xylose, and maltose under salinity stress (Gayen *et al.*, 2019). In a recent study, metabolic analysis of rice indicated high levels of sucrose and mannitol, and lower contents of quinate and shikimate in response to salinity stress (Chang *et al.*, 2019). Various metabolomics tools such as GC-MS, LC-MS, and NMR are being used to study metabolic variations in different crop plants like tomatoes, wheat, barley, rice, and maize (Rouphael *et al.*, 2018).

### Waterlogging Stress Regulation

Water logging affects crop plants growth and yield. In water logging stress, there is decrease in amount of CO<sub>2</sub> and oxygen which affects the photosynthesis mechanism. Water logging stress for a longer period result in decrease amount of CO<sub>2</sub> by affecting roots of the plants (Barding *et al.*, 2013). In an experiment, rice cultivars resilience was investigated under waterlogging stress. NMR- and GC/MS-based integrated metabolomics tools detected some unique metabolites, including phosphogluconate, phenylalanine, and lactate, that allowed rice plants to tolerant waterlogging stress conditions (Locke *et al.*, 2018).

### Heat Stress Regulation

Temperature changes have the potential to do serious harm and disrupt the developmental and plant normal growth process. Under extreme heat, bread wheat

untargeted metabolomics was carried out utilising LC-HRMS. This profiling revealed a significant increase in the production of metabolites such as pipercolate and L-tryptophan. Additionally, increased temperature has an impact on the production of aminoacyl-tRNA and plant secondary metabolites (Thomason et al., 2018). Using LC-MS/MS-HPLC techniques, the metabolic characterization of bread wheat grains after heat stress revealed greater amounts of sucrose and G1p (Wang et al., 2018).

### Concluding Remarks & Future Outlook

In modern biological research the field of metabolomics has accomplished a valuable place. Because it has ability to be applicable in various fields of plant sciences like cell mechanisms and their responses to numerous stresses. In recent era, it is allowing to improve the key properties of plants quality, yield, shelf life by assessing their pathways. The integration of modern tools with omics approaches in bioinformatics adeptly analyze novel metabolic networks for crop betterment.

In future metabolic markers identifications can enhance the plant metabolic pathways detection. It can improve the capability of a breeder in order to plan and develop superior plants that adequately fulfil the modern era needs. It can be associated with speed breeding and like other approaches for rapid research purpose.

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## Utilisation of Herbal Bullets against Newcastle Disease in Poultry Sector of Asia and Africa (2012-2022)

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### ABSTRACT

Poultry industry is rapidly booming in developing countries with increased demand for consumption. Reportedly, chicken meat is highly expected to be used as a major source of protein. With rising popularity and expansion comes greater risk to health and economic losses due to lethal diseases like New Castle disease. Such diseases not only make flocks vulnerable to morbidity but also increases mortality risks for the whole flock. Thus, controlling, managing, and treating these diseases is direly needed. Developing countries, for example, Asian and African countries, rely on a number of control methods and treatments. One of these is the use of ethnoveterinary medicine. Many researchers have also tested the efficacy of these medicines in various terms. In Pakistan, *Azadirachta indica*, *Nigella sativa*, *Glycyrrhiza glabra*, *Iresine herbstii*, etc. have been found effective against Newcastle Disease. *Phyllanthus embolic*, *Curcuma long* and *Ocimum tenuiflorum*, *Allium cepa*, *Cuminum cyminum*, *Withania somnifera*, *Tinospora cordifolia*, *Allium sativum*, *Azadirachta indica*, *Trigonella foenum-graecum* and *Laurus nobilis* are also found effective against ND in India. China is the largest poultry industry in the world that has also been employing various ethnoveterinary medicines for disease control in farms. Some of the most used ethnoveterinary medicines include *Astragalus membranaceus*, *Angelicae sinensis extract* and *Danggui Buxue San*, *Scutellaria baicalensis*, *Chinese plant*, *ginseng* (Chinese plant) stem-leaf saponins, *Rheum rhabarbarum*, *Glycyrrhiza glabra*, *Sijunzi Decoction*, *Dangguibuxue Tang* and *Morus alba* with vaccine adjuvant. In African countries, Ethnoveterinary botanical medicines are also used. The use of *Allium sativum*, *Azadirachta indica*, *Allium sativum with NDV lasota vaccine*, *Aloe barbadensis miller*, *Moringa oleifera*, *Lagenaria breviflora*, *Cucumis metuliferus*, *Piper guineense*, *Aframomum melegueta*, and *Psidium guajava* are known to be effective. Survey-based studies in Ethiopia have shown that *Acmella caulirhiza*, *Zingibar officinale*, Mixture of Alcohol, Citrus limon and *Allium cepa*, *Capsicum annum*, *Rumex abyssinicus* root and *Brasica compestris* are used as ethnoveterinary. Similarly, In Zimbabwe, *Capsicum annum*, *Sesamum angustifolium fruit*, *Tridactyle bicaudata leaves*, *Strychnos cocculoides*, *Senna singueana leaves*, *Abyssinia bark*, *Aloe greatheadii pods*, and other aloe species are used effectively against NDV. This literature review will highlight some herbs and ethnoveterinary medicines that have been researched to be effective against Newcastle Disease.

**Key words:** Ethnoveterinary Medicine, Botanicals, Asian Herbs, African herbs, Newcastle Disease.

### INTRODUCTION

Poultry is the high demanding agricultural sub-sector, particularly in developing countries, and a crucial key to alleviating poverty and catering income (Mottet & Tempio, 2017). When considering per unit of human intake, poultry meat, and eggs are major protein sources compared to mutton, beef, and milk and, are forecasted to be the highest consumed animal protein items in the next 2-3 years. In the

past decades, a marked increase in poultry consumption has been seen i.e. 16% globally with a projection of a further 5.5% soar in the forthcoming years (Das & Samanta, 2021).

Though, poultry still represents a threat to human health because of infectious diseases (viz. Newcastle disease, Avian Influenza, coccidiosis, etc.) (Mottet & Tempio, 2017). The transmission of these infectious diseases also causes serious impacts on the economic value of the country (Wang et al., 2013). Among these infectious

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diseases, Newcastle disease is a highly contagious and lethal disease even, the birds that suffer from this disease die without exhibiting any signs and symptoms and, infect lots of domestic and wild bird species. It is caused by virulent strains of Newcastle disease virus (NDV) or Avian Paramyxovirus -1, which is a single-strand non-segmented negative sense RNA virus. This NDV virus is prone to high mortality, morbidity, and loss in egg production in poultry flocks that results in economic losses. Epizootics of this disease occurs worldwide including Asia and Africa (Abdisa & Tagesu, 2017; Ansori & Kharisma, 2020). So, there is a dire need to control this disease.

Since Ancient times, use of natural resources like animal products, minerals, and plants have been done to treat many diseases of animals and humans. Millions of people prefer botanical medicines as primary health care (Korkmaz et al., 2016). People believe that these plant-based medicines are safer and cheaper than conventional therapy, therefore, ethno-veterinary botanical medicine use has been increasing for a long time now (McGaw et al., 2020). Another major reason for the increased use of these botanical medicines is the unavailability of modern veterinary medicines (Maphosa & Masika, 2010) and due to antibiotic resistance (Abdisa & Tagesu, 2017; Ansori & Kharisma, 2020). A lot of research data is also available which shows that plant-based home remedies are used to treat livestock diseases in Asian and African countries i.e. Pakistan (Abdisa & Tagesu, 2017; Aziz et al., 2020), India (Sikarwar & Tiwari, 2020), China

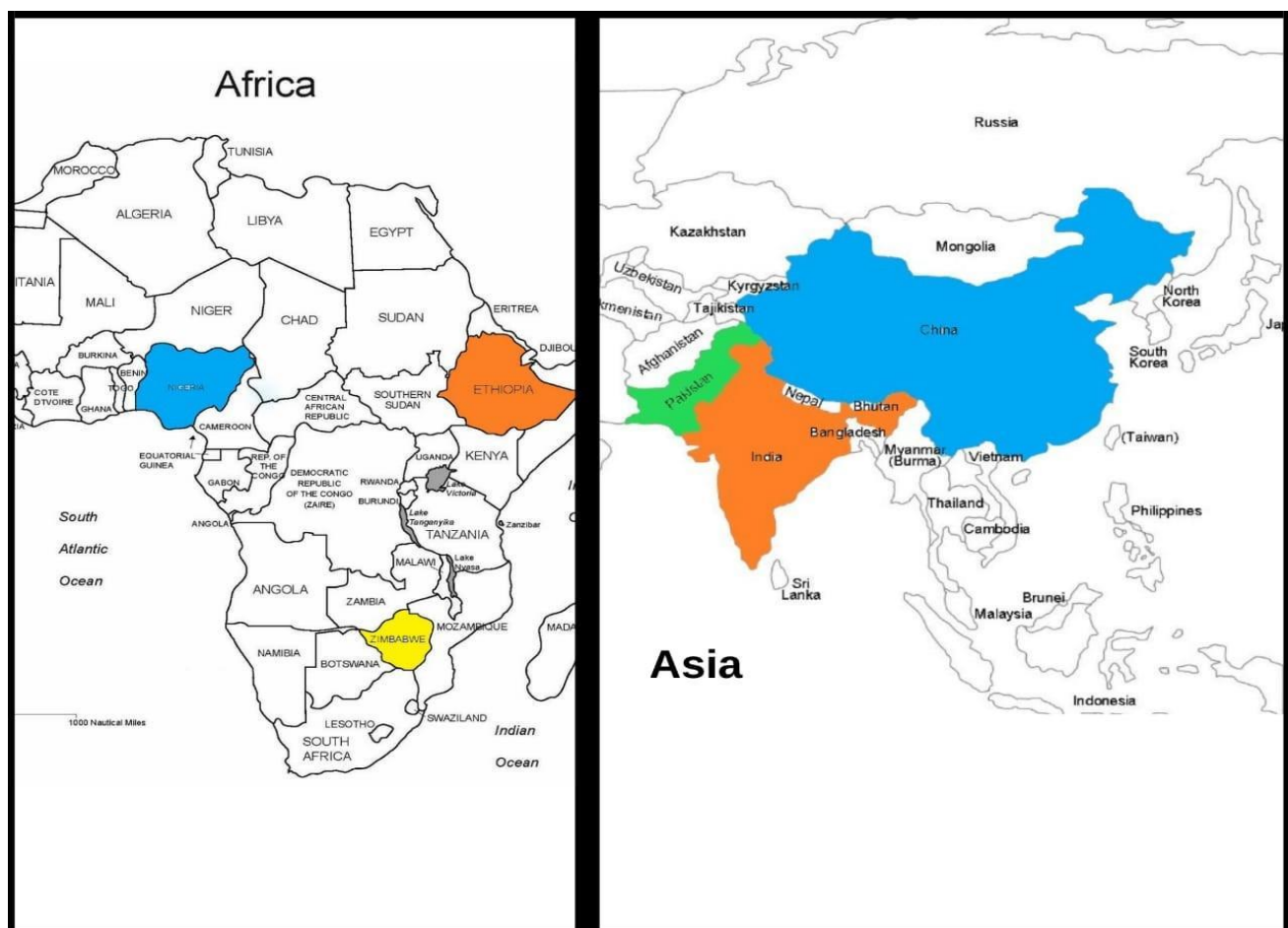
(Xiong & Long, 2020), Nigeria (Onwubiko et al., 2020), Ethiopia (Berhanu et al., 2020) and Zimbabwean (Jambwa & Nyahangare, 2020), etc. The literature said, 50-70 thousand plant species are used nowadays for the treatment of different animal diseases (ERARSLAN & Kültür, 2019).

In this review, we will discuss plant-based medicine used against Newcastle disease (cited in table 1) in different countries of Asia and the African continents during 2012-2022 as shown in Figure 1.

## MATERIALS AND METHODS

In this entire study, Google scholar was used as a main search engine. Moreover, other websites like science direct and research gate were as well used to search papers for the "Control of Newcastle disease by Ethno-veterinary botanical medicine". Furthermore, we took 6 different countries of Asia and Africa (Pakistan, India, China, Nigeria, Ethiopia, and Zimbabwe) and keywords that were used to search papers on this topic are "Newcastle disease", "Ethno-veterinary practice for the Newcastle disease", "Newcastle disease effects on poultry industry", "Ethno-veterinary practice against livestock and poultry diseases", "Evaluation of plant against Newcastle disease" in 6 different countries since 2012 to 2022.

A small number of review papers are used as parent articles. Neither quantification data nor statistical comparison has been done.



**Fig. 1:** Study areas to assess the use of ethnoveterinary botanical medicines used against ND.

## Literature Review

### Newcastle Disease and its Ethno-medicinal Treatment in Asian Countries:

#### I. Pakistan:

Pakistan is an agricultural land and integral part of its economy radically based on livestock and poultry. Every fifth family of this country is linked with the poultry industry and emerged as the 2<sup>nd</sup> largest industry of the country with a 4 percent annual increase, supplying eggs and meat. Since the launching of the organized poultry sector in Pakistan, ND caused serious destruction several times to the poultry sector (Shabbir et al., 2013). Most Pakistani farmers are poor and they cannot afford modern allopathic drugs for the control of infectious diseases of livestock, thus ethnoveterinary medicine is used for control and treatment (Murad et al., 2014). The plant products that are naturally produced play a vital role in the control and management of the disease (Mahmood et al., 2018).

*Azadirachta indica* (Neem), commonly found in Pakistan, belongs to the family Meliaceae is used against Newcastle disease. Neem has certain chemical and physical properties to fight against this deadly virus. In 2015, research has been done at Agricultural University, Faisalabad, in which Neem bark extract was used to evaluate its antiviral property. *In-vitro* evaluation was done by spot assay and micro-heamagglutinin test and *in-ovo* by injecting extract in 11-day-old embryonated eggs. The results described that Neem has a direct relation with antiviral activity to control ND (Mahmood et al., 2018).

*Nigella sativa* (Black seed) is an important botanical medicine used to treat different maladies of animals. Another research was conducted at University of Veterinary and Animal Science, Lahore in Pakistan to evaluate its antiviral effect against ND. In this research, ethanol was used to get the extract of black seed which was then inoculated in 14-days-old embryonated chick egg. Gross and histopathological examination revealed that it has a strong immunotherapeutic effect against ND (Khan et al., 2018).

*Glycyrrhiza glabra* (liquorice) has been traditionally used for the treatment and control of various infectious diseases. In 2017, research was conducted at GC university, Faisalabad, to evaluate this plant against NDV. Nine-day-old embryonated chick was inoculated with 5 different plant extracts, in which *Glycyrrhiza glabra* had powerful antiviral properties against NDV (Ashraf et al., 2017). In another research, *Glycyrrhiza glabra* (Licorice) was used again NDV with a combination of purified immunoglobulins. In this research, newly hatched broiler chicken was treated with this extract and it was concluded that purified immunoglobulins and Licorice have the potential to fight against ND (Amjad et al., 2022).

*Iresine herbstii* (blood leaf) is used as a food coloring agent. In 2019, this plant was tested against NDV and inoculated in 9-day-old chicken eggs. The results revealed that it has the potential to work against NDV because of its photochemical profile (Andleeb et al., 2020).

Some exclusive plant species found in the Cholistan region of Pakistan near the Cholistan Institute of Desert Studies, Baghdad ul Jadeed Campus, and the Islamia University of Bahawalpur have also been studied. The researcher evaluated 11 different plant species i.e. *Achyranthes aspera*, *Haloxylon recurvum*, *Haloxylon*

*salicornicum*, *Oxystelma esculentum*, *Octhocloa compressa*, *Neurada procumbens*, *Panicum antidotale*, *Salsola baryosma*, *Suaeda fruticosa*, *Sporobolus icolados* and *Solanum surattense* against NDV (Shahzad et al., 2019). Their extracts were made and inoculated in 7-11 days old embryonated chicken eggs. The results exhibit that *Achyranthes* was found most effective and *Oxystelma esculentum*, the least effective for the control of ND (Shahzad et al., 2019).

Another study showed a number of plants that can fight against NDV. Among these, the plants which showed *in vitro* antiviral activity are *Momordica balamina*, *Artemisia annua*, *Adansonia digitata*, *Azadirachta indica*, *Moringa oleifera*, and *Psidium guajava*, and, plants that showed *in-ovo* are *Cucumis metuliferus*, *Anthocleista nobilis*, and *Aloe secundiflora*. (Raza et al., 2015) And the plants which have the potential to improve the immune system of animals are *Nigella sativa*, *Mangrove halophytes*, *Melissa officinalis*, *Polysavone*, *Momordica cochinchinensis*, *Withania somnifera*, *Asparagus Racemosus*, and *Echinacea purpurea* (Raza et al., 2015). Hence, the use of ethnoveterinary medicine is common in Pakistan for the control of various diseases including Newcastle disease.

#### II. India:

In India, poultry is one of the swift growing sectors in the agriculture area and contributes about 1 percent of the Indian GDP (Pica-Ciamarra et al., 2011). Backyard poultry plays a significant role to alleviate poverty in the country. Yet, a lot of infectious diseases cause major loss, among these ND is one of the deadliest diseases and endemic in this country. This disease not merely wipe out the entire flock of poultry but is also a major constraining factor in the growth of the poultry industry (Dey et al., 2014).

India is rich in plant biodiversity but a very small amount of systematic studies has been done to evaluate plants against infectious diseases, and few pieces of research have been available for the treatment and control of infectious diseases (Shrivastava & Jain, 2016).

*Phyllanthus embolic* (amla), *Curcuma long* (turmeric), and *Ocimum tenuiflorum* (tulsi) have been used as herbal medicine and have a direct effect on ameliorating the mounting against ND (Reddy et al., 2012). In 2016, a seed mixture of *Phyllanthus amarus* (amla), *Allium cepa* (onion), and *Cuminum cyminum* (Cuminum, jera) was given orally for 3 days and results indicated that ND can be cured by these botanical medicines (Ahamad et al., 2016). Similarly, *Phyllanthus amarus*, *Cuminum cyminum* seeds, and *Allium cepa* pulp along with jaggery was used as an antiviral agent against ND in 2018 (Mekala et al., 2018). The research was conducted at Veterinary University Training and Research Centre, TANUVAS, Tirupur, Tamil Nadu. In this research, a grounded mixture of these plants was used orally at the rate of 10 g per liter for 5 days. The results concluded that these plants have potential activity against NDV (Mekala et al., 2018).

*Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi), *Allium sativum* (Garlic), and *Azadirachta indica* (Neem) were used against NDV without affecting the egg production and egg qualities. Results concluded with positive results of these plant mixtures (Mohanambal et al., 2018). In another study, *Allium sativum* (Garlic), *Trigonella foenum-graecum*

(fenugreek), and *Laurus nobilis* (bay leaves) herbal feed were used in laying hen to check the immunity status after giving these supplements. The results revealed that the combination of these herbs conferred a high level of immunity as well as a high level of immunoglobulins in eggs (Raj et al., 2013).

Another study aimed to use an herbal mixture of *Curcuma longa*, *Coriander sativum*, *Allium sativum*, *Andrographis paniculata*, and *Trigonella foenum graceum* against NDV. The outcome revealed positive results of this mixture (Priya et al., 2022). Hence, the ethnoveterinary medicines proved to be effective against NDV in India are amla, turmeric, tulsi, neem, onion, garlic, guduchi, bay leaves, and fenugreek.

### III. China:

China has the largest and most rapidly growing poultry industry and this plays a paramount role in the economy of the country (Tarique et al., 2013). Transmission of infectious diseases is a prone serious threat to the poultry sector of China (Wang et al., 2013). Newcastle infectious diseases is endemic in China for decades (Liu et al., 2008). Chinese herbal medicine plays a crucial role to control various infectious diseases not only humans sides but also in veterinary science (Lin & Panzer, 1994). There are a lot of vaccines that have been made in China by Chinese plants against NDV.

*Astragalus membranaceus* (AM) (common name: huáng qí or milkvetch), *Angelicae sinensis* (known as dong quai) extract, and Danggui Buxue San (Chinese herbal mixture) were used for the study of immunity, and hematinic mechanism of chicken against ND by giving these plants orally in the diet. The result revealed improvement in the haemopoietin function and immunity in Chicken (C. Li et al., 2013). Baicalin extract from *Scutellaria baicalensis* (Chinese skullcap) was used to investigate anti-NDV activities. In this study, Chicken Embryo Fibroblasts were infected with NDV and quantitative analysis of apoptotic cells was performed through cell cytometry. Cytotoxicity and antiviral activity of baicalin were found via MTT (3-(4, 5-dimethylthiazolyl)-2)-2, 5-diphenyltetrazolium bromide) assay method. The results revealed a positive impact of baicalin against NDV (Jia et al., 2016).

In 2019, ginseng (Chinese plant) stem-leaf saponins (GSLs) in combination with selenium (Se) were evaluated against NDV and IBV in chicken via an intraocular-and-intranasal route. The results depicted that the GSLs-Se group had the highest antibody response against NDV and IBV virus. Further in 2020, the effect of sunflower oil along with ginseng C.A Meyer plant's extract saponins were used to check the immune response of Chicken against Newcastle diseases. The results showed that it is safer against this deadly virus (Yuan et al., 2020).

Rhein derived from the traditional Chinese plant *Rheum rhabarbarum* (Rhubarb) was evaluated to see its antiviral potential against NDV. The results revealed that they effectively inhibited NDV activity and could be used as a future antiviral drug against NDV (Hu et al., 2022). *Glycyrrhiza glabra* (licorice) is a medicinal plant having antiviral properties because its chemical components include polysaccharides, flavones, and saponins. The polysaccharides extract of this plant was used in one

research that was performed on 14-day-old chick. And the results depicted that polysaccharides extracted from this plant have the ability to enhance the immune activity induced by NDV vaccine (Wu et al., 2022). Sijunzi Decoction is a Chinese herbal mixture and its components were used orally for the control of NDV. The results revealed that this mixture has strong capacity to enhance immunity in chickens and is safe with no adverse effect on the weight and survival of chickens (Zhanga et al., 2012).

Dangguibuxue Tang (DBT) Chinese herbal mixture is also used in one research to enhance immunity against ND virus and IBD virus. The result showed that 0.5% of DBT can ameliorate both cellular and humoral immunity of immunosuppressive chicken (X. T. Li et al., 2013).

Mulberry leaf polysaccharide was used against ND to provide evidence as a potential mucosal vaccine adjuvant (Chen et al., 2021). One study used 25 different medicinal herbs to investigate the cytopathic effect of NDV. Among these, 5 herbs that have the highest inhibitor effect are used including *Herba agastaches*, *Flos chrysanthemi indic*, *Rhizoma anemarrhenae*, *Astragalus root*, and *Baikal skullcap root*. The results suggested that formulation components have a synergistic effect to improve NDV resistance (Wang et al., 2016). These are some researches on Chinese herbal medicine in recent years for the control and treatment of the New Castle Disease Virus. Hence, Chinese herbal mixture, Chinese skullcap, licorice, DBT, Rhubarb, and Chinese plant are effective against ND in China.

### Newcastle Disease and its Ethno-medicinal Treatment in African Countries:

#### I. Nigeria:

Nigeria is one of the largest countries in Africa and makes one of the fastest growing economies in the world. The poultry industry in Nigeria has an important role in enhancing the economy of the country. This industry is rapidly expanding in recent years (Heise et al., 2015). Poultry infectious diseases are a major threat to the poultry industry and result in severe economic losses (Akintunde & Adeoti, 2014). ND is one of the deadliest infectious diseases in poultry that causes devastating effects on the economy of the country by high mortality and morbidity (Nwanta et al., 2008). In order to control these infectious diseases, ethnoveterinary botanical medicines are used to treat these diseases (Guèye, 1999). Some research has been done in Nigeria to treat NDV via plant-based medicine.

The aqueous extracts of *Allium sativum* (Garlic) and *Azadirachta indica* (Neem) leaf extract are used against NDV. 161-day-old broilers were treated with Garlic and Neem extract individually and their mixture. The research findings suggested that both Garlic and Neem have the capabilities to enhance the immunomodulatory response of the NDV vaccine (Garba et al., 2013).

Another research has been done to check the effect of *Allium sativum* (Garlic) and NDV Lasota vaccine, the birds were administered random treatment in their drinking water. The results revealed that *Allium sativum* (Garlic) and NDV lasota vaccines both have the potential to enhance the immune system of chickens against NDV (Bulus & Zaro, 2019). In 2015, the aloe vera plant was used to test the immune activity against NDV. 140 day old chicks were treated orally with aloe vera extract and results indicated

that this extract is the best immunomodulator against NDV (Ojjezeh & Ophori, 2015).

Moreover, the aqueous seed extract of *Moringa oleifera* (Zogale, horseradish tree) was used against NDV. 9-day-old chick was treated with this plant extract in one research and the results depicted that this plant has antiviral properties. (Chollom, Agada, Gotep, et al., 2012). In another study, *Lagenaria breviflora* (folklore medicine of West Africa) was used in rats. The results concluded that this medicine also has antiviral properties (Adedapo et al., 2013).

*Cucumis metuliferus* is commonly known as African horned cucumber, its alkaloids are administered in embryonated egg, and concluded that this plant pulp has a safer margin and good antiviral (Chollom, Agada, Bot, et al., 2012) activity against NDV (Anyanwu et al., 2016). Moreover, *Piper guineense* extracts also has properties against NDV. In one study, This plant extract was mixed in the drinking water of 48-dayold chick. The result showed that this plant has great potential to control NDV (Osho et al., 2016).

*Aframomum melegueta* (Guinea pepper) leave extract was inoculated in 10-day-old embryonated egg to evaluate the antiviral properties against NDV, fowl-pox virus, and infectious bursal disease. The results concluded with a positive response against these infectious diseases (Dike). One survey revealed that bitter leaf (*Vernonia amygdalina*) and Christmas melon (*Cucumis melo*) serve as a cure to prevent ND(Irivboje et al., 2021). Another botanical plant used to control NDV is *Psidium guajava* (apple guava), their leaf extract has nutritional value and antiviral properties against this deadly NDV. The research was conducted on a hatched chick. The results showed that it is a good immune booster against viruses (Chollom, Agada, Bot, et al., 2012). Hence, in Nigeria, various parts of different plant-based medicines are effective against NDV.

## II. Ethiopia:

More than 70% of Ethiopians are dependent on the livestock industry which showed a major share in the economy of this country. But, livestock diseases are the main constraint in the progress of the country. So, there is a need to control them (Lulekal et al., 2014). For the poultry industry of Ethiopia, ND causes serious destruction in this industry and is endemic in Ethiopic regions (Mazengia, 2012). Ethnoveterinary botanical medicines are used to control diseases in livestock (Lulekal et al., 2014). But the very limited amount of research has been done for the evaluation of veterinary botanical medicine against ND in Ethiopia. Some of the research are mentioned below;

A survey-based study has been conducted to identify ethnoveterinary medicine against poultry diseases. In which, *Acmella caulirhiza* Del. Plant leaves were used against NDV (Moliso et al., 2016). Another study has been

done to identify different plant species used against various infectious diseases of livestock. When we talk only for ND so, *Zingibar officinale* plant rhizome was used to treat NDV (Wodegebriel et al., 2018). Another survey was performed in 2017, in which alcohol, lemon, and white onion mixture was used to treat NDV (Bogale et al., 2017). Another study depicted that *Capsicum annum* (red pepper) seed mixed with oil in feed showed the potential for the treatment of NDV. Moreover, an aqueous extract of *mekmeko* root in drinking water was used against NDV. Furthermore, the aqueous extract of *Brasica compestris* (mustard seed) was also used against NDV and was found effective to combat NDV (Endalew et al., 2018). So, Ethiopia also employs ethnoveterinary medicine to protect poultry from various diseases including ND.

## III. Zimbabwe:

Poultry industry is a crucial sector for the livelihood of Zimbabwean natives. Farmers raise poultry for food and as a source of income. Hence, outbreaks of infectious diseases are prone to a great loss in society (Gobvu et al., 2022). Ethnoveterinary practice is part of the management of various diseases of animals but it is still an emerging field in Zimbabwe. The amount of literature regarding ethnoveterinary practices in Zimbabwe is found to be less in number. (Jambwa & Nyahangare, 2020).

In 2021, a research-based survey was conducted on avian botanical medicine in some selected communal areas of Zimbabwe. The study showed that 36 plant species are useful for the treatment of various poultry diseases. Plants that were used for ND are *Agave sisalana* perrine (mukonji, chickwengu), *Aloe chabaudii* (gavakava), *Aloe greatheadii*, *Bobgunnia madagascariensis* (snake bean pods), *Erythrina Abyssinia* bark, *Tridactyle bicaudata* leaves (paka), *Capsicum frutescene* (Toronga, mumhiripiri, chili pepper) (Jambwa et al., 2021). Hence, any of these ethnoveterinary medicines can be used against ND in one way or the other.

A systematic review has been done in 2022 about ethnoveterinary medicine for the treatment of different poultry diseases. So, the plants that are found in this study to treat NDV are *Capsicum annum* seed (crushed and mixed with sugar in drinking water), *Sesamum angustifolium* fruit (crushed and added to the drinking water), *Tridactyle bicaudata* leaves (pound the plant and suspended them in water), *Strychnos cocculoides* fruit (used in drinking water), *Senna singueana* leaves (used in drinking water), *Erythrina Abyssinia* bark (used in drinking water), *Bobgunnia madagascariensis* snake bean pods (used in drinking water) and aloe species (*Aloe vera*, *Aloe spicata*, *Aloe greatheadii*, *Aloe chabaudii*, *Aloe barbadensis*, and *Aloe arborescens*) (Gobvu et al., 2022).

**Table 1:** Ethno-veterinary medicines used in different countries

Sr. No.	Ethnoveterinary medicines/ Plants used (Scientific Name)	Common Names	Method of usage	Country of Research	Disease(s)	Reference(s)
1	<i>Azadirachta indica</i>	Neem	<i>In-vitro</i>	Pakistan	ND	(Mahmood et al., 2018)
2	<i>Nigella sativa</i>	Black seed	<i>In-ovo</i>			(Khan et al., 2018)
3	<i>Glycyrrhiza glabra</i>	Liquorice	<i>In-ovo</i>			(Amjad et al., 2022)

4	<i>Iresine herbstii</i>	Blood leaf	<i>In-ovo</i>		(Andleeb et al., 2020)
5	<i>Achyranthes aspera</i> , <i>Haloxylon recurvum</i> , <i>Haloxylon salicornicum</i> , <i>Oxystelma esculentum</i> , <i>Ochocloa compressa</i> , <i>Neurada procumbens</i> , <i>Panicum antidotale</i> , <i>Salsola baryosma</i> , <i>Suaeda fruticosa</i> , <i>Sporobolus icolados</i> and <i>Solanum surattense</i>	Various herbs	<i>In-ovo</i>		(Shahzad et al., 2019)
6	<i>Momordica balamina</i> , <i>Artemisia annua</i> , <i>Adansonia digitata</i> , <i>Azadirachta indica</i> , <i>Moringa oleifera</i> and <i>Psidium guajava</i>	Herbal mixture	<i>In-vitro</i>		(Raza et al., 2015)
7	<i>Cucumis metuliferus</i> , <i>Anthocleista nobilis</i> and <i>Aloe secundiflora</i>	African horned cucumber, Cabbage, African aloe vera	<i>In-ovo</i>		(Raza et al., 2015)
8	<i>Nigella sativa</i> , Mangrove halophytes, <i>Melissa officinalis</i> , <i>Polysavone</i> , <i>Momordica cochinchinensis</i> , <i>Withania somnifera</i> , <i>Asparagus Racemosus</i> and <i>Echinacea purpurea</i>	Various herbs	<i>In-ovo</i>		(Raza et al., 2015)
9	<i>Phyllanthus embolic</i> , <i>Curcuma longa</i> and <i>Ocimum tenuiflorum</i>	Amla, Turmeric, Tulsi	Per Oral (mixed with feed)	India	(Reddy et al., 2012)
10	<i>Phyllanthus amarus</i> , <i>Allium cepa</i> and <i>Cuminum cyminum</i>	Amla, Onion, cuminum, Jera	Per Oral (mixed with feed)		(Ahamad et al., 2016)
11	<i>Phyllanthus amarus</i> , <i>Cuminum cyminum</i> seeds and <i>Allium cepa</i> pulp along with jaggery	Carry me seed, Cumin, Onion	Per Oral (mixed with water)		(Mekala et al., 2018)
12	<i>Withania somnifera</i> , <i>Tinospora cordifolia</i> , <i>Allium sativum</i> and <i>Azadirachta indica</i>	Ashwagandha, guduchi, Garlic, Neem	Per Oral		(Mohanambal et al., 2018)
13	<i>Allium sativum</i> , <i>Trigonella foenum-graecum</i> and <i>Laurus nobilis</i>	Garlic, Fenugreek, Bay leaves	Per Oral (mixed with feed)		(Raj et al., 2013)
14	<i>Curcuma longa</i> , <i>Coriander sativum</i> , <i>Allium sativum</i> , <i>Andrographis paniculata</i> , and <i>Trigonella foenum graceum</i>	Various herbs	<i>In-ovo</i>		(Priya et al., 2022)
15	<i>Astragalus membranaceus</i> , <i>Angelicae sinensis</i> extract and Danggui Buxue San	Huáng qí or Milkvetch, Dong quai, and (Chinese herbal mixture)	Per Oral	China	(X. T. Li et al., 2013)
16	<i>Scutellaria baicalensis</i>	Chinese skullcap	<i>In-vivo</i>		(Jia et al., 2016)
17	Ginseng stem-leaf saponins in combination with selenium	Chinese plant	intraocular-and-intranasal route		(Ma et al., 2019)
18	Sunflower seed oil Ginseng C.A. Meyer (E515-D)	Chinese plant	Per-oral		(Yuan et al., 2020)
19	<i>Rheum rhabarbarum</i>	Rhubarb	Per-oral		(Hu et al., 2022)
20	<i>Glycyrrhiza glabra</i>	Licorice	<i>In-ovo</i>		(Wu et al., 2022)
21	<i>Sijunzi Decoction</i>	Chinese herbal mixture	Per-oral		(Zhanga et al., 2012)
22	Danggui Buxue Tang	Chinese herbal mixture	<i>In-vitro</i>		(X. T. Li et al., 2013)
23	<i>Morus alba</i>	Mulberry leaf polysaccharide	Vaccine adjuvant		(Chen et al., 2021)
24	<i>Herba agastaches</i> , <i>Flos chrysanthemi indic</i> , <i>Rhizoma</i>		<i>In-vivo</i>		(Wang et al., 2016)

	<i>anemarrhenae</i> , Astragalus root and Baikal skullcap root				
25	<i>Allium sativum</i> and <i>Azadirachta indica</i>	Garlic, Neem	Per-oral	Nigeria	(Garba et al., 2013)
26	<i>Allium sativum</i> and NDV lasota vaccine	Garlic	Per-oral		(Bulus & Zaro, 2019)
27	<i>Aloe barbadensis miller</i>	Aloe Vera plant	Per-oral		(Ojiezeh & Ophori, 2015)
28	<i>Moringa oleifera</i>	Zogale, Horseradish tree	Per-oral		(Chollom, Agada, Gotep, et al., 2012)
29	<i>Lagenaria breviflora</i>	Folklore medicine of West Africa			(Adedapo et al., 2013)
30	<i>Cucumis metuliferus</i>	African horned cumcumber	<i>In-ovo</i>		(Chollom, Agada, Bot, et al., 2012)
31	<i>Piper guineense</i>	Ashanti pepper	Per-oral(mixed with drinking water)		(Anyanwu et al., 2016)
32	<i>Aframomum melegueta</i>	Guinea pepper	<i>In-ovo</i>		(Osho et al., 2016)
33	<i>Vernonia amygdalina</i> and <i>Cucumis melo</i>	Bitter leaf Christmas melon			(Dike)
34	<i>Psidium guajava</i>	Apple guava	Per-oral		(Irvineboje et al., 2021)
35	<i>Acmella caulirhiza</i>	Toothache plant or paracress	Per-oral	Ethiopia	(Chollom, Agada, Bot, et al., 2012)
36	<i>Zingibar officinale</i>	Ginger	Per-oral		(Moliso et al., 2016)
37	Alcohol, <i>Citrus limon</i> , <i>Allium cepa</i>	Alcohol, lemon, and white onion	Per-oral		(Wodegebril et al., 2018)
38	<i>Capsicum annum</i>	Red pepper	Per-oral (mixed with feed)		(Bogale et al., 2017)
39	<i>Rumex abyssinicus</i> root	Mekmeko	Per-oral (mixed with drinking water)		(Endalew et al., 2018)
40	<i>Brasica compestris</i>	Mustard seed	Per-oral		(Endalew et al., 2018)
41	<i>Agave sisalana perrine</i> , <i>Aloe chabaudii</i> , <i>Aloe greatheadii</i> , <i>Bobgunnia madagascariensi</i> , <i>Erythrina Abyssinia bark</i> , <i>Tridactyle bicaudata</i> leaves, <i>Capsicum frutescene</i>	Mukonji, Chickwengu, Gavakava, Snake Bean pods, paka, Toronga, mumhiripiri, Chili pepper	Per- oral (mixed with water)	Zimbabwe	(Jambwa et al., 2021)
42	<i>Capsicum annum</i> seed, <i>Sesamum angustifolium</i> fruit, <i>Tridactyle bicaudata</i> leaves, <i>Strychnos cocculoides</i> fruit, <i>Senna singueana</i> leaves, <i>Erythrina Abyssinia bark</i> , <i>Bobgunnia madagascariensis</i> and aloe species ( <i>Aloe vera</i> , <i>Aloe spicata</i> , <i>Aloe greatheadii</i> , <i>Aloe chabaudii</i> , <i>Aloe barbadensis</i> and <i>Aloe arborescens</i> )	Various botanical materials and herbs	Per-oral		(Gobvu et al., 2022)

## Conclusion

Worldwide, Newcastle disease is creating havoc in terms of economic losses. To curb the disease, the use of ethnoveterinary medicine is much more economical and easy to use in all countries, especially developing countries. In this review, 6 different countries are studied that have done research on the use of ethnoveterinary medicine. Out of these countries, the majority are developing countries. In

Asian countries, Pakistan and China have done more research on ethnoveterinary medicines as compared to India. Pakistan has studied Neem, Black seed, Liquorice, Blood leaf, Herbal mixture, African horned cucumber, Cabbage, and African aloe vera as ethnoveterinary medicines and these proved to be effective against Newcastle disease. The use of Huáng qí or milkvetch, Dong quai, Chinese herbal mixture, Chinese skullcap,

Chinese plant, Rhubarb, Licorice, and Mulberry leaf polysaccharide in China against Newcastle disease was found successful. India successfully researched the use of Amla, Turmeric, Tulsi, Onion, Cuminum, Jera, Carry me seed, Ashwagandha, Guduchi, Garlic, Neem, Fenugreek, and Bay leaves for the prevention and control of Newcastle disease. Similarly, the African countries included in the study utilized ethnoveterinary medicine which are; Garlic, Neem, Aloe vera plant, Zogale, horseradish tree, Folklore medicine of West Africa, African horned cucumber, Ashanti pepper, Guinea pepper, Bitter leaf, Christmas melon, Apple guava in Nigeria; Toothache plant or paracress, Ginger, Alcohol, lemon, White onion, Red pepper, Mekmeko, and Mustard seed in Ethiopia; Mukonji, Chickwengu, Gavakava, Snake bean pods, Paka, Toronga, Mumhiripiri, and Chili pepper in Zimbabwe were researched and found to be effective against Newcastle disease. The number of research found in Nigeria was relatively more than the other two African countries and Zimbabwe was found to have the least number of research done. The use of such medicines against Newcastle disease in these countries must be done more thoroughly to have sufficient data for deriving conclusions regarding their uses. Moreover, there is a need of studying various parts of plants in more detail to use the most bioactive part of plants against NDV.

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## Assessment of Cassava Peel/Palm Kernel Cake Meal (PKM) on Growth Performance and Blood Parameters of Lactating Sows (Agricultural Extension Implication)

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### ABSTRACT

A four weeks study was carried out to assess the effect of cassava peel/palm kernel cake meal on the growth characteristics of lactating sows, piglets and blood parameters. Forty large white lactating sows with average weight (72-78kg) and piglets mean weight range from 27 to 35kg were randomly distributed to the five treatments in a completely randomized design (CRD). The combination was attained on one to one weight basis. The combinations replaced maize at 0 (control), 10, 20, 30 and 40%. Feed/water were made readily available. The results on performance characteristics, hematological parameters and serum biochemical parameters fed difference inclusion of cassava peel/PKC meal were significantly ( $P<0.05$ ) influenced. Performance of lactating sows recorded a higher weight loss 30% (18.13kg) and piglet mortality 40% (14.81%), while piglet average weekly weight gained (7.5kg) and weaned (30) was better in 10%. However, this study recommended 10% level of cassava peel/pkc meal in lactating sow feed.

**Key words:** Growth Performance, Lactating Sow, Piglets, Cassava Peel/Palm Kernel Cake, Blood Parameters

### INTRODUCTION

Nigeria is among the highest populated black race in the world and ranked first in Africa. However, the country is not growing along with its population in areas of world technological advancement that will translate into food production that will meet up needs of its populace used to calculate the growth domestic product. Nigeria has continuously dropped in the world ranking today due to inadequate channeling or total neglect of agriculture most especially livestock (Moseri et al., 2020). In Nigeria, Agriculture accounts for 35% of GDP before the predominance of oil, and major earner of foreign currency. Now the oil is on a decline, there is a great clamor for diversification of Nigerian economy. This has to redirect attention to Agriculture and now is the time for government of Nigeria to pay unprecedented attention to agricultural development that will serve as an instrument in reducing hunger, malnutrition and starvation (Amaza et al., 2021). Livestock have been an important subsector of agriculture in Nigeria contributing about 1.24 trillion and fishing 384.4 billion naira respectively. Furthermore, engaging about 35% of the country's population; the sector has equally

been a major provider of animal protein, thereby making a significant contribution to national nutritional security. There is dearth between the population and food production in our country today due to kidnapping, banditries and insecurity that is forcing most of the farmer out of production (Moseri et al., 2020). However, efforts should be channel in the utilization of locally available agro waste materials such as brewery dried grains, palm kernel waste, etc. in other to reduce a stiff competition currently between human and animals for conventional grains, to salvage these problems being encountered by farmers most especially piggy farmers. Hence cassava peel and palm kernel cake meal will serve as pivotal that will help to ameliorate this problem today. Thus, the objective of this study was to assess the effect of cassava peel and pkc meal on the performance characteristic and blood parameters of lactating sows.

### MATERIALS AND METHODS

#### Experimental Site

The study was done at research farm, Faculty of Agriculture, Ambrose Alli University, Ekpoma, Edo state Nigeria.

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### Source of Test Ingredients

The cassava peel and palm kernel cake were sourced from garri factories and open markets within Agbor town. Other constituents were procured from renowned animal feed dealers in Benin City and its neighborhoods.

### Investigational Animals Design, Housing and Management

The research farm is an improved Danish type of housing, with fundamental passage and exposed bodybuilding yards, the main structural sorts of the house is low walls of 1.2m, over which are timber frames and supporting roof. The roof was made up of asbestos, ideal for heat shield and protection from the effect of direct solar emission and control of thermal tension. Pigs were housed on a concreted floor pen, each of which had a concrete inherent water trough and feeding cubicles. A total of 40 gilts average weight (72-78kg) of large white and mean initial weight of piglets (27-34kg) were distributed into 5 groups created on regular initial weight in a Completely Randomized Design (CRD). The lactating sows were fed double daily and water supplied *ad libitum*. The treatment diets contained a mixture of sundried cassava peels and PKC (in a 1:1 ratio, w/w) at 0, 10, 20, 30 and 40% respectively. The 1:1 ratio of cassava peel to palm kernel cake was derivative by mixing equivalent weights (kg) of the two test constituents in the diet using a manual scale. All diets were formulated to be iso-nitrogenous and iso-caloric in Table 1. The experiment lasted for four weeks of post natal sow.

### Data Collection

Feed intake and weight gain were recorded, a known quantity of feed was supplied and the corresponding left over recovered and measured. The difference between what was supplied and the left over divided by the time

intermission is the daily feed intake. Pigs from each level were weighed at the commencement and subsequently weekly to determine the body weight. The dissimilarity between the initial and final body weight is the weight gain viz; average piglet birth weight (kg), average piglets weaned weight (kg), number of piglet's weaned weight (kg), weight loss during lactation (kg) and mortality rate. Animals were deprived of feed for 12 hours before blood samples were taken from each pig from the ear vein via a sterilized disposable syringe and needle. Preceding to bleeding, a cotton swab soaked in 70 % ethanol was used to clean the ear vein and to inhibit infection or impurity of the blood sample. A 5.0ml blood was taken from each pig into branded sterilized bottles comprising Ethylene-Diamine-Tetra-Acetic acid (EDTA) as anticoagulant, were used to determine the total red blood cells (RBC), hemoglobin (Hb), and packed cell volume (PCV) and white blood cell (WBC). Another 5.0 ml of blood was collected into labeled sterile sample bottles without anticoagulant were used to determine the serum biochemical components of total protein, albumin, globulin and serum cholesterol.

### Data Analysis

Data were analyzed with SAS (2003) package, and differences amongst treatment means were parted using Duncan's multiple range test (1955) as defined by Obi (2002).

## RESULTS

Performance characteristics of lactating sows is shown in Table 2. Average final weight loss value of lactating sow was lowest in control diet (63.78kg) and increased as an increasing rate of the diets of 10 (60.50), 20 (58.29), 30 (55.55) and 40% (53.13kg) respectively. Significant ( $P < 0.05$ ) differences was noted in average total weight

**Table 1:** Composition of experimental diet for lactating sows fed cassava peel/pkc meal

Ingredients	0	10%	20%	30%	40%
Maize	40.00	36.00	32.00	28.00	24.00
Cassava peel/PKC	-	04.00	08.00	12.00	16.00
Ground Nut Cake	15.87	17.00	18.13	19.26	20.39
Wheat Offal	38.18	36.05	34.72	33.39	32.06
Bone Meal	1.50	1.50	1.50	1.50	1.50
Limestone	2.00	2.00	2.00	2.00	2.00
Palm Oil	1.00	2.00	2.20	2.40	2.60
Fattener Premix*	0.25	0.25	0.25	0.25	0.25
Salt	0.35	0.35	0.35	0.35	0.35
Ronozyme**	0.20	0.20	0.20	0.20	0.20
Lysine	0.65	0.65	0.65	0.65	0.65
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis:					
Crude Protein (%)	16.92	16.54	16.36	16.69	16.87
ME(Kcal/Kg)	2770.86	2774.00	2734.92	2758.48	2737.44
Fat (%)	4.58	6.20	7.8	9.44	11.09
Fiber (%)	5.03	6.66	8.28	9.98	11.51
Ash (%)	5.91	9.01	12.11	15.3	18.53
Calcium (%)	0.80	0.80	0.80	0.80	0.80
Starch (%)	41.00	37.79	34.58	31.34	28.08

\*Vitamin-mineral premix/kg diet: Vitamin A-8,000 IU, Vitamins D3 -3,000 IU, Vitamins E-8 IU, Vitamin K-2mg, Vitamin B1- 1 mg, Vitamin B2-0.2 mg, Vitamin B12-5 mg, Nicotinamide-10 mg, Selenium- 0.1 mg, Ca Pantothenate - 5 mg, Folic acid -0.5 mg, Choline Chloride-150 mg, Iron -20 mg, Manganese -80 mg, Copper -8mg, Zinc -50 mg, Cobalt -0.225mg, Iodine -2 mg Antioxidant - 0.1ppm Key:- CPM = Cassava peels meal, PKC = Palm kernel cake, GNC = Groundnut cake, C.P. = Crude protein, ME = Metabolizable energy.

\*\*Ronozyme Composition: sodium sulfate (52.7%), calcium carbonate (15%), kaolin (9%), dextrin and sucrose (8%), cellulose (6%) and vegetable oil (7%).

loss of lactating sows, highest weight loss value was recorded for 40% (18.13kg) and the least weight loss value was in 0% (14.22kg). Lactating sows average weekly feed intake ranged from 34.90kg (40%) to 43.74kg (20%) in the experimental diets.

Performance characteristics of piglets at 28 days after farrowing is shown in Table 3. Total weight of the piglet values ranges from 27.68kg (40%) to 31.04 kg (0%) fed cassava peel/pkc meal. Significant ( $P<0.05$ ) differences were documented in total weight gain, number of piglets weaned, average weekly weight gain and mortality percentage at weaned. Final total weight gain of piglets value was highest in 10% (225.00kg) and lowest in 40% (100.25kg), while the average weekly weight gain was better in 10% (7.50kg) compared to 0% (7.25kg), 20% (6.75), 30% (5.75kg) and 40% (5.05kg) with least gain.. Number of piglets had the highest value in 10% (30.00kg) and least value was in 40% (22.00kg). However, mortality percentage recorded was highest in 30% (14.81) and lowest in 40% (3.25).

Hematological parameters of lactating sows shown in Table 4. Result shows a significant ( $P<0.05$ ) difference in packed cell volume (PCV) values of 37.70, 37.50, 49.50, 43.48 and 41.82 % were recorded for 0, 10, 20, 30 and 40 % diets respectively. Red blood cell (RBC) had the highest value of  $15.65 \times 10^{-6}/\text{ml}$  (0 %) diet with the lowest value of  $6.05 \times 10^{-6}/\text{ml}$  (40 %) sows. White blood cell (WBC)

recorded values of 13.10, 20.20, 9.60, 15.60 and  $19.40 \times 10^{-3}/\text{ml}$  for sows fed 0, 10, 20, 30 and 40 % diets respectively. Hemoglobin was highest in 20 % (14.50g/dl), and lowest value in 40% (10.80g/dl). Mean corpuscular volume (MCV) value ranged from 65.60 to 69.20(g/dl). Mean corpuscular hemoglobin (MCH) had the highest in 40% (20.80pg) and least value was observed in 30% (19.70pg). Mean corpuscular hemoglobin concentration (MCHC) values ranges from 29.90 (0%) to 30.10 % (20%).

Serum biochemical parameters of lactating sows is presented in table 5, total protein, albumin, globulin, creatinine, urea, cholesterol and glucose significantly ( $P<0.05$ ) differed in the diets. Total protein value was highest in 0 % (13.49g/dl), followed by 30 (9.30), 10 (8.75), 40 (8.64) and 20% (8.60) g/dl with lowest value. Albumin values of sows fed diet 0, 10, 20, 30 and 40 % were 5.08, 3.57, 4.35, 4.58 and 4.49 g/dl separately. Globulin values decreased as the stages of cassava peels/pkc diet increased with obtained values of 8.41, 5.18, 4.25, 4.72, and 4.15 (g/dl) for 1, 2, 3, 4 and 5 diets. Creatinine values ranges from 1.97 to 2.93 (g/dl). Urea diets of 0, 10, 20, 30 and 40 % with values of 61.12, 36.23, 45.98, 44.39 and 31.25 (g/dl) respectively. Cholesterol values ranged from 40% (64.80mg/dl) to 0% (130.39mg/dl). Glucose values were 0 (68.96), 10 (74.69), 20 (69.15), 30 (69.96) and 5 (60.75mg/dl) in that order.

**Table 2:** Performance of lactating sows fed cassava peel/pkc meal

Parameters	Levels of Inclusion (%)					SEM ( $\pm$ )
	0	10	20	30	40	
Average initial weight of lactating sow (kg)	78.00 <sup>a</sup>	77.50 <sup>a</sup>	73.00 <sup>bc</sup>	74.00 <sup>b</sup>	72.00 <sup>c</sup>	0.98
Average final weight loss of sow (kg)	63.78 <sup>a</sup>	60.50 <sup>b</sup>	58.25 <sup>c</sup>	55.55 <sup>d</sup>	53.13 <sup>e</sup>	0.25
Average weight loss of sow (kg)	14.22 <sup>d</sup>	17.00 <sup>bc</sup>	14.75 <sup>d</sup>	18.00 <sup>ab</sup>	18.13 <sup>a</sup>	0.35
Average total feed intake (kg)	275.25 <sup>a</sup>	262.50 <sup>b</sup>	225.74 <sup>c</sup>	225.74 <sup>c</sup>	210.00 <sup>d</sup>	0.15
Average weekly feed intake (kg)	42.88 <sup>b</sup>	43.74 <sup>a</sup>	41.13 <sup>c</sup>	37.63 <sup>d</sup>	34.90 <sup>e</sup>	0.22

a,b,c,d,e means along the same row with different superscripts are significant different from each other, SEM: Standard error of mean.

**Table 3:** Performance of piglets at 28 weeks after farrowing fed cassava peel/pkc meal

Parameters	Levels of Inclusion (%)					SEM ( $\pm$ )
	0	10	20	30	40	
Average initial weight of piglet (kg)	31.04 <sup>b</sup>	34.01 <sup>a</sup>	31.90 <sup>b</sup>	29.70 <sup>c</sup>	27.60 <sup>d</sup>	0.08
Final total weight gain (kg)	210.25 <sup>b</sup>	225.00 <sup>a</sup>	182.25 <sup>c</sup>	132.25 <sup>d</sup>	100.25 <sup>e</sup>	0.15
Number of piglets weaned (kg)	28.00 <sup>bc</sup>	30.00 <sup>a</sup>	27.00 <sup>c</sup>	23.00 <sup>d</sup>	22.00 <sup>e</sup>	0.42
Average weekly weight gain (kg)	7.25 <sup>b</sup>	7.50 <sup>a</sup>	6.75 <sup>c</sup>	5.75 <sup>d</sup>	5.05 <sup>e</sup>	0.09
Mortality (%)	9.38	3.25	6.90	14.81	4.35	-

a,b,c,d,e means along the same row with different superscripts are significantly ( $P<0.05$ ) different from each other, SEM: Standard error of mean.

**Table 4:** Hematological parameters of the lactating sows fed cassava peel/pkc meal

Parameters	Levels of Inclusion (%)					SEM ( $\pm$ )
	0	10	20	30	40	
PCV (%)	37.70 <sup>d</sup>	37.50 <sup>d</sup>	49.50 <sup>a</sup>	43.48 <sup>b</sup>	41.82 <sup>e</sup>	0.02
RBC ( $\times 10^{-6}/\text{ml}$ )	15.65 <sup>a</sup>	5.56 <sup>e</sup>	7.18 <sup>b</sup>	6.67 <sup>c</sup>	6.05 <sup>d</sup>	0.02
WBC ( $\times 10^{-3}/\text{ml}$ )	13.10 <sup>d</sup>	20.20 <sup>a</sup>	9.60 <sup>e</sup>	15.60 <sup>c</sup>	19.40 <sup>b</sup>	0.04
Hb (g/dl)	11.70 <sup>c</sup>	14.25 <sup>ab</sup>	14.50 <sup>a</sup>	13.90 <sup>b</sup>	10.80 <sup>d</sup>	0.24
MCV (g/dl)	66.80 <sup>c</sup>	67.60 <sup>b</sup>	69.00 <sup>a</sup>	65.60 <sup>d</sup>	69.20 <sup>a</sup>	0.21
MCH (pg.)	20.00 <sup>b</sup>	20.10 <sup>be</sup>	20.70 <sup>a</sup>	19.70 <sup>b</sup>	20.80 <sup>a</sup>	0.21
MCHC (%)	29.90 <sup>c</sup>	29.80 <sup>d</sup>	30.10 <sup>b</sup>	30.20 <sup>a</sup>	30.10 <sup>b</sup>	0.03

a, b,c,d,e means along the same row with different superscripts are significantly ( $P<0.05$ ) different from each other, SEM: Standard error of mean.

**Table 5:** Serum biochemical parameters of lactating sows fed cassava peel/pkc meal

Parameters	Levels of Inclusion (%)					SEM ( $\pm$ )
	0	10	20	30	40	
Total protein (g/dl)	13.49 <sup>a</sup>	8.75 <sup>c</sup>	8.60 <sup>d</sup>	9.30 <sup>b</sup>	8.64 <sup>d</sup>	0.02
Albumin (g/dl)	5.08 <sup>a</sup>	3.57 <sup>e</sup>	4.35 <sup>d</sup>	4.58 <sup>b</sup>	4.49 <sup>c</sup>	0.02
Globulin (g/dl)	8.41 <sup>a</sup>	5.18 <sup>b</sup>	4.25 <sup>d</sup>	4.72 <sup>c</sup>	4.15 <sup>e</sup>	0.02
Creatinine (mg/dl)	2.93 <sup>a</sup>	2.87 <sup>b</sup>	1.97 <sup>d</sup>	2.75 <sup>c</sup>	1.97 <sup>d</sup>	0.02
Urea (mg/dl)	61.12 <sup>a</sup>	36.23 <sup>d</sup>	45.98 <sup>b</sup>	44.39 <sup>c</sup>	31.25 <sup>e</sup>	0.02
Cholesterol (mg/dl)	130.39 <sup>a</sup>	106.97 <sup>b</sup>	64.80 <sup>b</sup>	94.47 <sup>c</sup>	64.80 <sup>d</sup>	0.02
Glucose (mg/dl)	68.96 <sup>d</sup>	74.69 <sup>a</sup>	69.15 <sup>c</sup>	69.92 <sup>b</sup>	60.75 <sup>e</sup>	0.02

a,b,c,d,e means along the same row with different superscripts are significantly ( $P < 0.05$ ) different from each other, SEM: Standard error of mean.

## DISCUSSION

Results on performance characteristics of lactating sows fed varied inclusions of cassava peel/pkc meal based diets shown in Table 2, indicated that dietary treatments compete favorably in average weight loss of lactating sows falls within the recommended 20% body loss of sows during lactating period. The average final weight loss was highest in 40 % diet, this indicated that lactating sows fed 40% diet suffered more weight loss compared to other diets, while 20 % cassava peels/pkc performed better in the weight loss or weight decrease fed the diets translated more to the piglet weight. The results agreed with the conclusion of Borges et al. (2005) who recommended a weight loss of lactating sows between 11 – 22kg within a lactating period of 28 days. Average total feed intake and average weekly feed intake in the dietary treatments reduced with amplified stages of cassava peel/pkc meal in the diets. However, variation in feed intake noticed could be ascribed to sows that have a higher number of piglets which will consumed more feeds to meet up the required milk by the piglets which is in concomitant with Nielson et al. (2013), that posited small litters size consumed lesser feed compared to a higher litter sizes. The body weight of piglet, final total weight gain, number of piglet weaned and average weekly weight gain presented in Table 3, indicated that 10% diet have contributed higher individual growth rates compared to other diets because there is more milk available for each of the pigs in the litter and increase the survival rate of the piglets. This finding corroborates with Grez et al. (2016) in their study that reported a range of 6.20 – 7.00 kg piglet weight at 28 days. The birth weight depend on placental nutrient supply which is largely determined by placental size. Piglets weight at farrowed and weaned fall within recommended size by Jackson (2009) who posited a range of 0.8-1.6 kg for normal piglets weight at farrowed that support early weaning, high levels of survival and reduced loss of sow body mass. Mortality was highest at 30 % which can be attributed to the weaned weight that was below recommended weight by Grez et al. (2016). The significance of blood cannot be exaggerated as we all know that blood plays a vivacious role in the life of all living organisms. Blood can be used to measure the abnormality in cells which impair the primary physiological functions of the animal body. Hematological aberration is vital in determining how healthy an animal is as an evidence of the extent of toxicity and nutritional quality of the levels of inclusion of some important feed ingredients. The hematological values of lactating sows shown in Table 4, packed cell volume (PCV), white blood cell (WBC) and

Red blood cell (RBC) falls within recommended range of pigs. This indicated absence of infection and toxicity in lactating sows fed the experimental diets, compete favorable with the control and values were within the standard ranges reported by Moseri et al. (2020). When hematological values fall within the normal assortment recognized for the animal, is a sign that diet did not show any antagonistic effect during the study. Further agreed with Etim et al. (2014) posited that hematological behaviors especially PCV and Hb were associated with the nutritional status of animals. It was concluded that the diets were not destructive to the lactating sows. Serum biochemical parameters shown in Table 5, indicated that protein levels in the diet sustained standard protein reserves in lactating sows occasioning from effective protein consumption. It has proved that protein reserves of animals are influenced by dietary protein without any shortage or alterations of albumin content (Adesehinwa et al., 2011). Serum creatinine and urea levels shows that lactating sows ensured muscular efficient of diets. This result validated the report of Moseri et al. (2020).

## Conclusion

The study has showed that feeding of 10% cassava peel/pkc meal to a lactating sow compete famously with other diets in area of weight loss, piglets weaned and weight gain. It is therefore recommended to incorporate 10% of cassava peel/pkc meal into lactating sow feed.

## Agricultural Extension Implication

Agricultural extension service providers should target pig farmers in the study area with the view of educating them on the use of cassava peel/Pkc meal in the feeding of lactating sow. Emphases should be on 10% inclusion of cassava peel /PKC meal as it have proved to be a better replacement for maize in the feeding of lactation sow. This will help farmers to reduce the production cost expended from the use of conventional feed.

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